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The Role of Topoisomerase II in Meiotic Chromosome Condensation and Segregation in *Schizosaccharomyces pombe*

Edgar Hartsuiker,* Jürg Bähler,† and Jürg Kohli

Institute of General Microbiology, University of Bern, 3012 Bern, Switzerland

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Topoisomerase II is able to break and rejoin double-strand DNA. It controls the topological state and forms and resolves knots and catenanes. Not much is known about the relation between the chromosome segregation and condensation defects as found in yeast *top2* mutants and the role of topoisomerase II in meiosis. We studied meiosis in a heat-sensitive *top2* mutant of *Schizosaccharomyces pombe*. Topoisomerase II is not required until shortly before meiosis I. The enzyme is necessary for condensation shortly before the first meiotic division but not for early meiotic prophase condensation. DNA replication, prophase morphology, and dynamics of the linear elements are normal in the *top2* mutant. The *top2* cells are not able to perform meiosis I. Arrested cells have four spindle pole bodies and two spindles but only one nucleus, suggesting that the arrest is nonregulatory. Finally, we show that the arrest is partly solved in a *top2 rec7* double mutant, indicating that topoisomerase II functions in the segregation of recombined chromosomes. We suggest that the inability to decatenate the replicated DNA is the primary defect in *top2*. This leads to a loss of chromatin condensation shortly before meiosis I, failure of sister chromatid separation, and a nonregulatory arrest.

INTRODUCTION

In meiosis one round of DNA replication is followed by two nuclear divisions. The chromosome number is halved, and four daughter cells are formed. In meiotic prophase, after DNA replication, the homologous chromosomes pair and recombine. In the following two nuclear divisions the homologous chromosomes (meiosis I) and the sister chromatids (meiosis II) are segregated. In the great majority of sexually reproducing eukaryotes a meiosis-specific tripartite structure is formed during meiotic prophase: the synaptonemal complex (for review, see von Wettstein *et al.*, 1984). The fission yeast *Schizosaccharomyces pombe* is an exception; no synaptonemal complex is formed. During meiotic prophase so-called linear elements appear, which resemble the axial elements of other eukaryotes. These elements are formed discontinuously along the chromosomes and never form a tripartite structure (Olson *et al.*, 1978; Hirata and Tanaka, 1982; Bähler *et al.*, 1993). In meiotic prophase the nuclei become elongated and are called horse tail nuclei (Robinow, 1977). A striking nuclear movement takes place during this time, which is led by the spindle pole body with attached telomeres (Chikashige *et al.*, 1994).

Topoisomerase II is an enzyme that is able to break and rejoin double-strand DNA molecules. In this way, it can control the topological state of the DNA and form and resolve knots and catenanes in duplex DNA. Several results suggest that topoisomerase II is required for segregation of replicated circular and linear DNA sister molecules. It has also been shown that topoisomerase II is necessary for mitotic chromosome condensation. Other roles for topoisomerase II have also been suggested (for review, see Watt and Hickson, 1994; Koshland and Strunnikov, 1996).

For mitosis, the phenotypes as seen in *Saccharomyces cerevisiae* and *S. pombe* temperature-sensitive (ts)[1] *top2*

* Corresponding author. E-mail address: hartsuiker@imb.unibe.ch.
† Present address: Cell Cycle Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, UK.

[1] Abbreviations used: cs, cold sensitive; DAPI, 4′,6-diamino-2-phenylindole; ts, temperature sensitive.

mutants can be explained by defects in segregation and chromosome condensation. In both yeasts topoisomerase II is essential for viability (DiNardo *et al.*, 1984; Goto and Wang, 1984; Uemura and Yanagida, 1984). Synchronously dividing cells of ts *top2* mutants become inviable, when shifted to restrictive temperature, at the time of mitotic division (Holm *et al.*, 1985; Uemura and Yanagida, 1986). Different studies in *S. cerevisiae* and *S. pombe* suggest a role for topoisomerase II in the separation of sister chromatids. In ts *top2* mutants the cells arrest at the mitotic nuclear division. In these arrested cells plasmids are found in the form of catenated dimers (DiNardo *et al.*, 1984; Uemura and Yanagida, 1986). The mitotic arrest is nonregulatory in both yeasts. It was shown in *S. cerevisiae* that the lack of topoisomerase II activity leads to nondisjunction and chromosome breakage in mitosis (Holm *et al.*, 1989; Spell and Holm, 1994). In *S. pombe* a mitotic spindle is formed and tries to separate the sister chromatids. Then the septum appears and cuts the nucleus across (cut phenotype; Uemura and Yanagida, 1984, 1986). The stabilization of normally unstable large circular minichromosomes in *S. pombe* by overexpression of topoisomerase II is also consistent with a role of topoisomerase II in the segregation of replicated DNA daughter molecules (Murakami *et al.*, 1995). Uemura *et al.* (1987) showed that topoisomerase II is necessary for proper chromosome condensation in mitosis of *S. pombe*.

In meiosis, cold-sensitive (cs) *top2* cells of *S. cerevisiae* arrest at the first meiotic division at restrictive temperature. Premeiotic DNA replication, chromosome condensation during prophase, and recombination are not affected. Complementary temperature shift experiments suggest that the enzyme is only required at the time of the nuclear divisions (Rose *et al.*, 1990). The arrest has been interpreted to be regulatory. The spindle pole bodies do not separate, and no spindle is formed; the arrested cells are able to return to mitotic growth. The cs *top2* cells seem to be delayed in the exit from pachytene (Rose and Holm, 1993). In a *top2 rad50* double mutant, in which recombination is blocked and no synaptonemal complex is formed, the cells are able to perform the first but not the second meiotic division (Rose *et al.*, 1990). Because of the dual phenotype of *rad50,* two hypotheses about the nature of the arrest were put forward. The first one is that topoisomerase II is needed for the segregation of recombined chromosomes (Rose *et al.*, 1990). The second one is that topoisomerase II functions in the resolution of interlocks that occur during the course of homologous chromosome pairing. The synaptonemal complex might play a role in detecting the unresolved interlocks and triggering the regulatory arrest (Rose and Holm, 1993).

A number of questions still remain to be answered about the role of topoisomerase II in segregation and chromosome condensation. It is not clear whether or how the defects in chromosome segregation and condensation are related. Especially about the role of topoisomerase II in meiosis not much is known. Therefore we studied meiosis in topoisomerase II deficient fission yeast, a widely used eukaryotic model organism.

MATERIALS AND METHODS

Strains

The diploid strains used in this study are h^+/h^- *ade6-M216/ade6-149* (Bähler *et al.*, 1993; in this paper referred to as wild type), h^+/h^- *top2-191/top2-191 leu1-32/leu1-32 ade6-M216/ade6-M210* (referred to as *top2*), and h^+/h^- *top2-191/top2-191 rec7-102/rec7-102 leu1-32/leu1-32 ade6-M210/ade6-M210* (referred to as *top2 rec7*). The *top2-191* allele was isolated by Uemura and Yanagida (1984). The *rec7-102* allele was isolated by Ponticelli and Smith (1989). For strain construction, standard genetic methods were used (Gutz *et al.*, 1974).

Culture Conditions and Meiotic Time Courses

The cells were cultured and shifted to meiosis-inducing medium as described by Bähler *et al.* (1993). Permissive and restrictive temperatures of 24 and 34°C, respectively, were used for meiotic time courses. Because after shift to meiotic medium the cells first have to perform a mitotic division (which is lethal at restrictive temperature for the *top2* and *top2 rec7* strains), the cultures were first incubated for 2.5 h at 24°C and then divided in two cultures, which were incubated at 24 and 34°C, respectively, for the rest of the time course (but not for the complementary temperature shift experiment; see below). At different time points after shift to meiosis-inducing conditions samples were taken and processed for spreading and 4′,6-diamino-2-phenylindole (DAPI) staining as described below. Samples for immunofluorescence were taken and processed as described below.

Complementary Temperature Shift Experiments

In the first experiment (see Figure 1A) *top2* and wild-type diploid cells were shifted to meiotic conditions and incubated at restrictive temperature. At hourly intervals aliquots were removed and shifted to permissive temperature. At the same time cells were fixed for DAPI staining, and the percentage of cells that completed the first meiotic division was determined. Sporulation efficiency was determined the next day. In the second experiment (Figure 1B), after shift to meiotic conditions, cells were incubated at permissive temperature, and every hour an aliquot was removed and shifted to restrictive temperature. The rest of the experiment was performed as described for the first experiment. For both experiments, at least 100 cells were counted from every time point for determination of the sporulation efficiency and percentage of cells that performed the first meiotic division.

Nuclear Spreading, DAPI Staining, and Immunofluorescence

Nuclear spreading and DAPI staining were performed as described by Bähler *et al.* (1993). After staining with silver nitrate and transfer to grids, nuclear spreads were examined by electron microscopy with a Philips EM300 at 60 kV (Bähler *et al.*, 1993). For each time point at least 100 nuclei were analyzed. DAPI staining was performed as described by Bähler *et al.* (1993), and at least 100 cells per time point were analyzed by fluorescence microscopy. For immunofluorescence double staining the two primary antibodies were incubated simultaneously, as were the secondary antibodies. The rabbit polyclonal SAD1 antibody against the spindle pole body (Hagan and Yanagida, 1995) was isolated and generously provided by I. Hagan (University of Manchester, United Kingdom). The monoclonal rabbit TAT1 antibody against microtubules (Woods *et al.*, 1989) was generously provided by K. Gull (University of

Manchester). For immunofluorescence we used the protocol as described by Hagan and Hyams (1988), with some alterations as described by Svoboda *et al.* (1995). As secondary antibodies affinity-isolated goat anti-mouse immunoglobulin G TRITC conjugate (Fab specific, Sigma T-6528) and affinity-isolated goat anti-rabbit immunoglobulin G FITC conjugate (whole molecule, Sigma F-0382) were used.

Analysis of DNA Replication and Chromatin Condensation

Cultures of wild type and *top2* were shifted to meiosis as described above. Cells were cultured for 2.5 h at 24°C and then shifted to 34°C.

For the analysis of DNA replication, every hour 1 ml of each meiotic culture was briefly centrifuged, resuspended in 1 ml 70% ethanol, and stored at 4°C. After staining the cells with propidium iodide, the DNA content of the cells was determined by flow cytometry (Beach *et al.*, 1985).

For the analysis of chromatin condensation, every hour a sample was taken and prepared for DAPI staining (see above). From each time point pictures were taken with a digital charge-coupled device camera on a fluorescence microscope and stored for further analysis. Exposure settings of the camera were identical throughout the experiment. For each time point, from at least 200 cells containing one nucleus the nuclear area and the total nuclear fluorescence (giving a measure of DNA content) were determined with the University of Texas Health Science Center (San Antonio, TX) ImageTool program (http://ddsdx.uthscsa.edu/dig/itdesc.html) using the same threshold settings throughout to define the outline of the nuclei. The total fluorescence as determined with this method is roughly linear to the DNA content of the nucleus, as suggested by the fact that G_2 nuclei give twice the fluorescence intensity of G_1 nuclei (our unpublished results). In the *top2* mutant, cells that showed the cut phenotype (see INTRODUCTION) were left out of the analysis. For each nucleus, the nuclear fluorescence was divided by the nuclear area, giving a measure of nuclear condensation.

RESULTS

Topoisomerase II Is Not Required during Meiotic Prophase but at the Time of the Meiotic Divisions

To determine at which time topoisomerase II is needed during meiosis, we performed two complementary temperature shift experiments (Rose *et al.*, 1990; also see Materials and Methods). The first experiment revealed from which time on topoisomerase II was needed for sporulation (Figure 1A). The sporulation efficiency of the wild-type strain stayed constant over time. However, the *top2* strain showed a strong decrease immediately after shift to restrictive temperature. This first decrease reflected that the majority of the cells had to perform a mitotic division, which was fatal at restrictive temperature, before they could start meiosis from G_1. Accordingly, cells with two nuclei showing the cut phenotype were apparent during this time (our unpublished results). Therefore, we interpreted this decrease as a result of mitotic inviability. After 2 h, the sporulation efficiency reached a plateau. As can be seen in Figure 1C, this corresponds to an increase in the amount of horse tail nuclei, typical for meiotic prophase, in wild type. A second decrease in sporulation efficiency took place after 5 h. This coincided with an increase in the number of wild-type cells that completed the first meiotic division and a decrease in the number of horse tail nuclei. Data about *top2* horse tail nuclei could not be presented in Figure 1C, because the high abundance of mitotic cells with an elongated nucleus attributable to the cut phenotype made an objective estimation impossible. The low percentage of *top2* cells containing more than one nucleus at later time points (Figure 1A) suggests that the cells arrest before the first meiotic division (also see below). A repetition of this experiment gave similar results (our unpublished results). From these experiments we concluded that topoisomerase II is first needed at the premeiotic division, and again at the time of the first meiotic division, but not during meiotic prophase.

The complementary experiment (see MATERIALS AND METHODS) showed from which time on topoisomerase II was no longer needed for sporulation (Figure 1B). Again the sporulation efficiency of the wild-type strain stayed constant over time. The *top2* strain showed an increase in sporulation efficiency at ~8 h. This coincided with an increase in the number of *top2* cells that performed a meiotic division and a decrease in the number of horse tail nuclei. From this experiment we concluded that topoisomerase II is not required for sporulation after the meiotic divisions.

DNA Replication and Chromatin Condensation

In *S. pombe* topoisomerase II is necessary for mitotic chromosome condensation (Uemura *et al.*, 1987; see INTRODUCTION). We studied DNA replication and meiotic chromatin condensation in wild-type and *top2* strains in a meiotic time course at 34°C. To compare meiotic DNA replication between wild-type and *top2* cells at restrictive temperature, we determined the DNA content of the cells by flow cytometry (see MATERIALS AND METHODS). For every hour the percentage of cells in G_2 was determined (Figure 2A). Beginning at 1 h there was a decrease in the percentage of G_2 cells, reflecting the mitotic division before the start of meiosis (see Figure 2E). After 6 h the percentage of G_2 cells increased again, reflecting meiotic DNA replication. The kinetics of DNA replication between wild type and *top2* was similar. At the end of meiosis the percentage of G_2 cells was somewhat lower in the *top2* mutant. This was probably because the analysis of DNA replication in the *top2* mutant was obscured by the contribution of arrested dead cells showing a G_1 DNA content (cut phenotype; see INTRODUCTION). The DNA content of the arrested *top2* cells (11 h) was similar to the mitotic G_2 DNA content (0 h), indicating that DNA replication is normal in *top2* (Figure 2C).

The size of a nucleus is determined both by its DNA content and its degree of chromatin condensation. To get a measure of the degree of chromatin condensation, we determined for every time point the nuclear

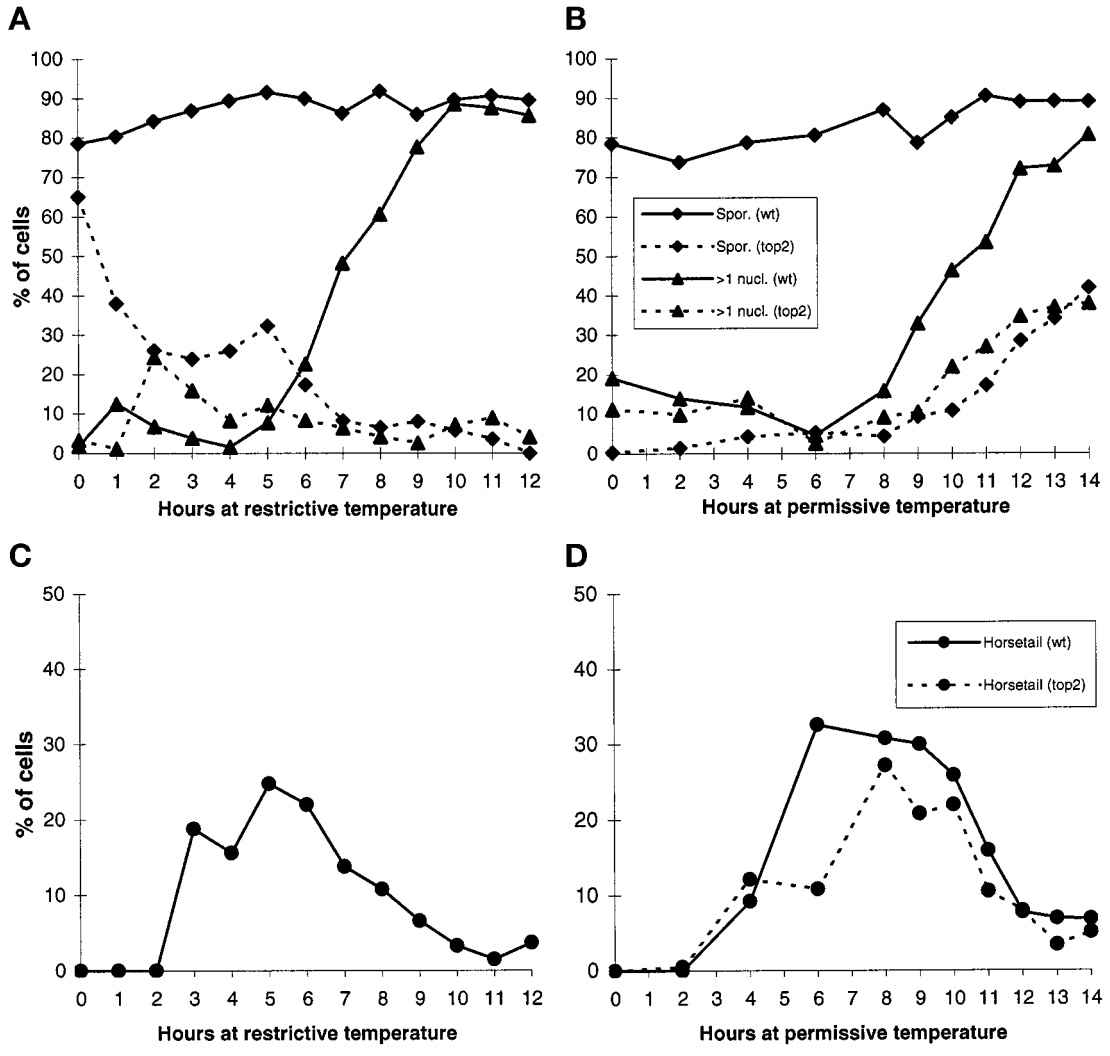
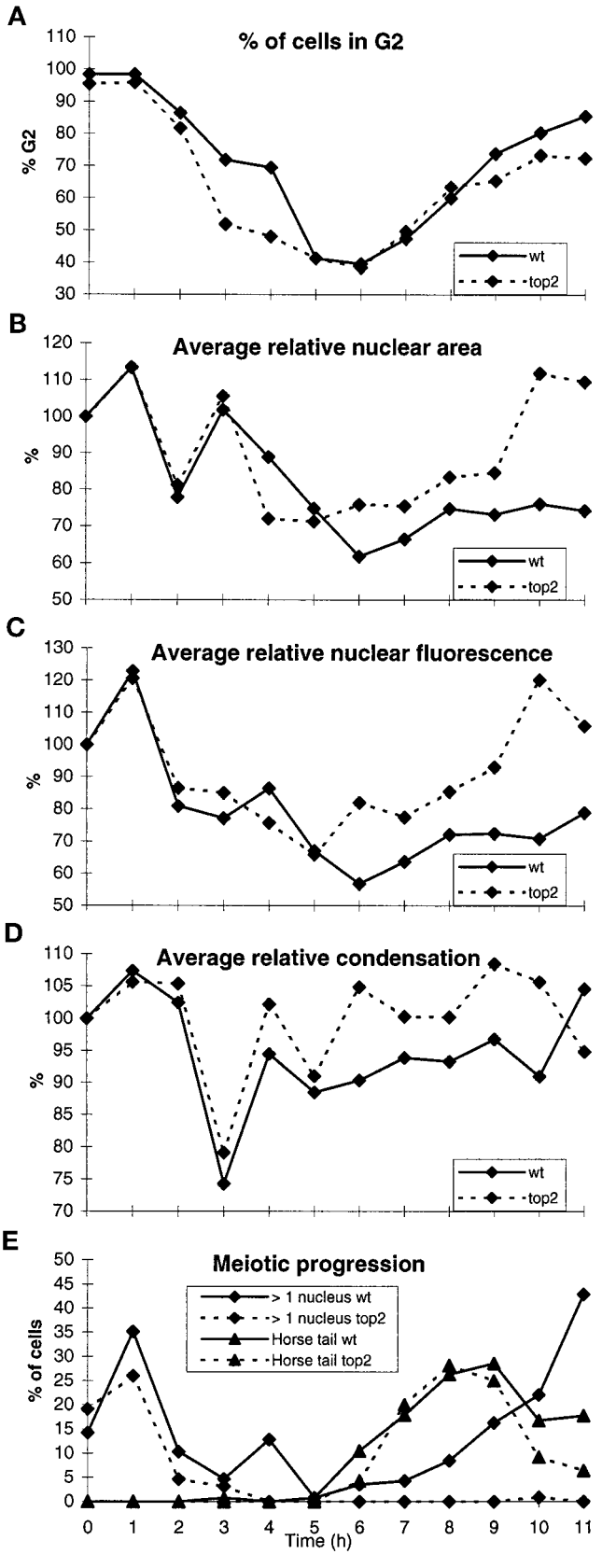


Figure 1. Complementary temperature shift experiment. For details see MATERIALS AND METHODS. (A) In the first experiment *top2* and wild-type (wt) cells were shifted to meiotic conditions and incubated at restrictive temperature. At hourly intervals aliquots were removed and shifted to permissive temperature. (B) In the second experiment, after shift to meiotic conditions, cells were incubated at permissive temperature, and every hour an aliquot was removed and shifted to restrictive temperature. (C) The percentage of horse tail nuclei for the experiment presented in A is shown for wild type. Data about horse tail nuclei in *top2* could not be included, because the high abundance of mitotic cells with an elongated nucleus caused by the cut phenotype made an objective estimation impossible. (D) Data about horse tail nuclei in *top2* as well as in wild type for the experiment presented in B are shown. In this experiment the first meiotic division was delayed in the *top2* strain compared with wild type at permissive temperature. The *top2* mutation has a slow growth phenotype at permissive temperature, probably because the cells have a problem with the mitotic division. The same problem probably delayed meiosis I in the *top2* mutant at permissive temperature. Spor., sporulation efficiency; >1 nucl., percentage of cells that performed the first meiotic division.

area and total nuclear fluorescence (measure of DNA content) of at least 200 cells with only one nucleus. A repetition of this time course experiment with formaldehyde-fixed cells gave similar results (our unpublished results). For details see MATERIALS AND METHODS. This method permits the quantitative analysis of large numbers of nuclei in a meiotic time course experiment. The preparation of the cells is simple and requires only a few steps. No harsh treatment, possibly creating artifacts, is needed, which is an advantage compared with studying condensation with in situ hybridization methods.

Determination of the nuclear area is shown in Figure 2B. The average values were standardized to the average nuclear area at 0 h (100%). At 2 h, for both wild type and *top2*, there was a sharp dip in the average relative area because of the mitotic division, which took place at 1 h (see Figure 2E) and reduced the DNA content of the cells. Then, for both strains, there was a remarkable increase in average relative area at 3 h.



This cannot be explained by an increase in DNA content (Figure 2, A and C). Then the average relative area decreased again to a level somewhat lower than at 2 h. Starting at 5 (*top2*) and 6 h (wild type) the average relative area started to increase slowly as a result of DNA replication (see Figure 2A). In wild type the average relative area reached a plateau at ~8 h. This was because the effect of DNA replication on the average relative nuclear area was counteracted by meiotic chromatin condensation shortly before the first meiotic division (see below; Robinow, 1977). In *top2* the average relative area increased at the end of the meiotic time course to a level somewhat higher than the mitotic average relative area at 0 h.

We also determined the total nuclear fluorescence of each of the cells, giving a measure of the DNA content (Figure 2C). Again the average values were standardized to the average nuclear fluorescence at 0 h (100%). At 2 h there was a sharp decrease in average relative fluorescence as a result of the transition of the cells from G_2 to G_1 because of the mitotic division (Figure 2, A and E). Starting at 5 (*top2*) and 6 h (wild type) the average relative fluorescence increased again, in *top2* to a level similar to mitotic (G_2) cells. In wild type the increase in DNA content was underestimated because of the progression of the replicated meiotic prophase cells into cells with two and four nuclei, which were left out of the analysis.

To get a measure of the degree of chromatin condensation, for each nucleus the nuclear fluorescence was divided by its nuclear area (Figure 2D). For each time point the average condensation was standardized to the average condensation at 0 h (100%). A sharp decrease in the average relative condensation was seen at 3 h. At 4 h the average relative condensation increased again to a level somewhat lower than at 0 h. At 11 h the average relative condensation in wild type increased, whereas it decreased in *top2*.

From the above observations we concluded that right after the mitotic division, a remarkable decondensation and recondensation takes place in both wild-type and *top2* cells. This decondensation and recondensation was also seen in wild-type meiosis at 30°C (our unpublished results) and appears to be independent of topoisomerase II. In wild type, at the end of meiotic prophase, the nuclei condense again

Figure 2. In a meiotic time course, DNA content, nuclear area, nuclear fluorescence, and chromatin condensation were determined in wild type and *top2* at 34°C. For detailed explanation see text and MATERIALS AND METHODS. (A) For every hour the percentage of cells that are in G_2 is shown. (B and C) The average relative nuclear area (B) and the average relative nuclear fluorescence (C) are shown for every hour. (D) The nuclear area divided by the DNA content gives a measure of chromatin condensation. (E) The timing of meiotic events in this particular time course is shown. wt, wild type; >1 nucleus, percentage of cells that performed the first meiotic division; Horse tail, percentage of cells that show the typical prophase nuclear morphology after DAPI staining.

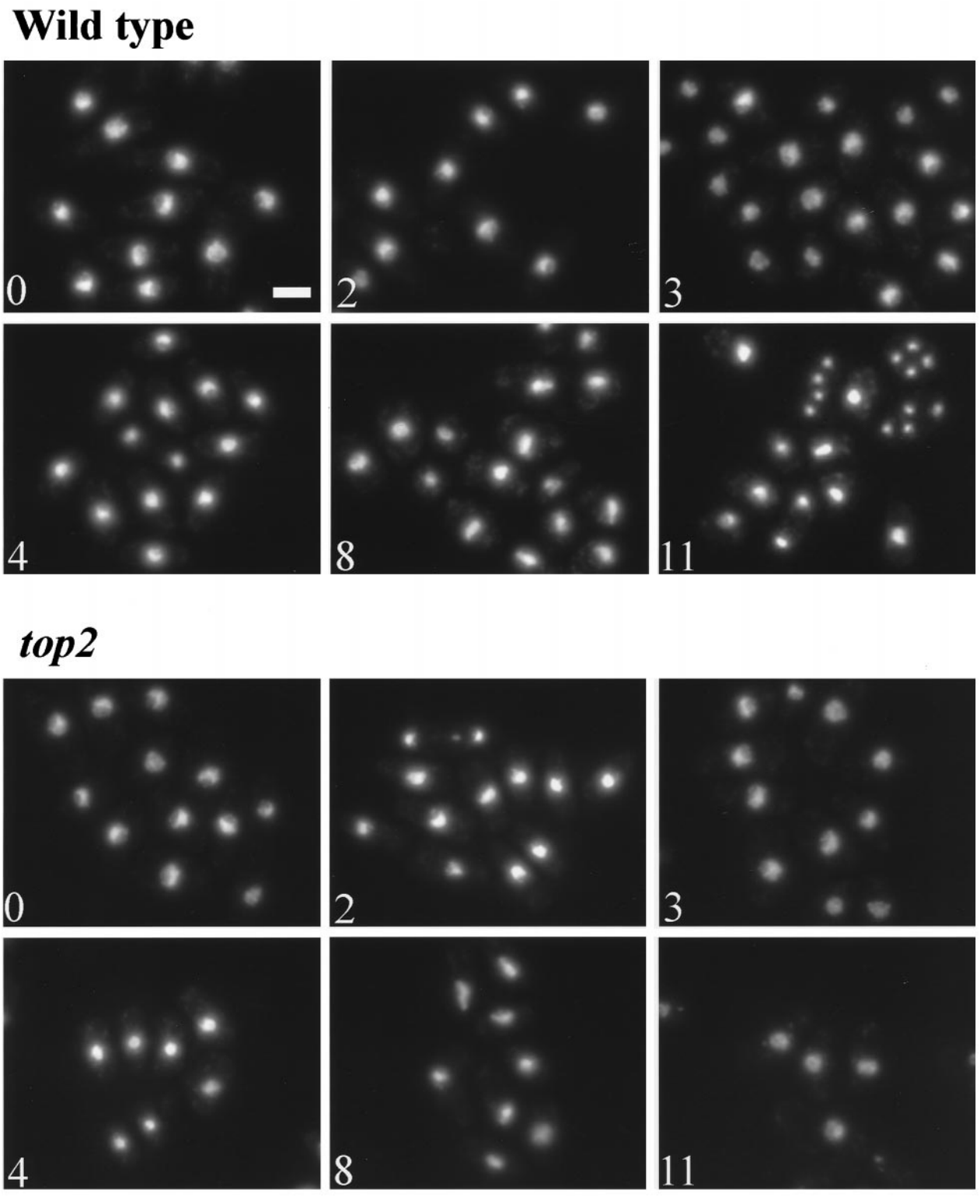


Figure 3. Illustration of the changes in chromatin condensation during meiotic time courses in wild-type and *top2* cells at 34°C. Quantitative data on these time courses are presented in Figure 2. At 0 h the majority of the still mitotic cells are in G_2 (also see Figure 2A) and show a dense nuclear staining. At 2 h the cells have performed a mitotic division (see Figure 2E), are smaller, and have a lower DNA content. The nuclei are densely stained, and the level of chromatin condensation is similar to that at 0 h (see Figure 2D). At 3 h the cells still have a DNA

(also see Robinow, 1977). This wild-type condensation was underestimated in the time course experiment, because it was immediately followed by meiosis I (cells with two or four nuclei were left out of the analysis). In *top2*-arrested cells (see below) the nuclei were not condensed at the end of the meiotic time course. Thus the condensation shortly before the first meiotic division is topoisomerase II dependent. These changes in chromatin condensation are illustrated in Figure 3. For explanation see figure legend.

Nuclear Structures in Meiotic Prophase Are Normal in top2 Cells at Restrictive Temperature

We performed three meiotic time course experiments in which we compared different characteristics between the *top2* and wild-type strains. In nuclear spread preparations no obvious differences in morphology of the linear elements were visible between the two strains at 24 and 34°C (our unpublished results). Also, the dynamics of appearance and disappearance of the linear elements was similar between the two strains at 24 and 34°C (Figure 4). An interesting observation, beyond the scope of this study, is that for both the wild-type and *top2* strains the percentage of linear-element containing cells was reduced at 34°C compared with 24°C. Because the sporulation efficiencies (>1 nucleus) of the wild-type strain were similar at 24 and 34°C, the decrease of linear elements might reflect the reduction in recombination frequency at 34°C compared with 25°C (Bähler *et al.*, 1991). Also the percentage of horse tail nuclei was reduced at 34°C compared with 24°C (Figure 4). By DAPI staining no obvious differences in morphology of the prophase nuclei (horse tail nuclei; see INTRODUCTION) were visible between the two strains at 24 and 34°C (our unpublished results).

The top2 Cells Arrest before the First Meiotic Division, but the Spindle Pole Body Cycle Continues

Although DNA replication was normal, and no clear phenotype was visible in meiotic prophase of the *top2* strain at restrictive temperature, the cells were not able to perform the first meiotic division. As can be seen in Figure 4D, the percentage of cells that contained more than one nucleus stayed close to zero at later time points.

In spread preparations of the arrested *top2* cells, often four spindle pole bodies were visible in one nucleus (Figure 5). As much as 27% of the nuclei showed this morphology at 7 h (Figure 6), but afterward the percentage decreased again. This was probably due to an overrepresentation of early cells late in the time course because of the poor spreading of advanced cells. At later time points at restrictive temperature, DAPI-stained *top2* cells were found with chromatin fibers stretched crosswise out of the nucleus (Figure 7, a and b). Because this suggested that the cells were actively trying to separate the chromatin, we also did a double-staining immunofluorescence experiment in whole cells with antibodies that recognize the spindle and spindle pole body, respectively. At 7 h a small portion of the meiotic cells showed two spindles and four spindle pole bodies (Figure 7c–e). The cell shown has four spindle pole bodies (Figure 7c) and two spindles (Figure 7d), probably representing both spindles of the second meiotic division. Only one nucleus is visible, with chromatin fibers stretching out along the spindle (Figure 7e). This striking morphology was never observed at permissive temperature or in the wild-type strain. The final arrest stage was also observed during 2 h in several living cells with video fluorescence microscopy. During this time the spindle attempted to segregate the chromosomes, because transient protrusions from opposite sides of the nucleus were observed (our unpublished results). Thus at restrictive temperature the spindle pole body cycle continues in the *top2* mutant, although the first meiotic division was not completed.

Arrest Is Partly Solved in a top2 rec7 Double Mutant

It has been proposed that topoisomerase II is required for the segregation of sister chromatids of recombined chromosomes during meiosis I (Rose *et al.*, 1990). To test this hypothesis in *S. pombe* we studied meiosis in a *top2 rec7* double mutant (see MATERIALS AND METHODS). In the *rec7-102* mutant, intergenic recombination is strongly reduced (DeVeaux and Smith, 1994), whereas the morphology and dynamics of the linear elements is similar to wild type (Molnar, personal communication). The morphology and dynamics of appearance and disappearance of the linear elements in the *top2 rec7* strain at permissive and restrictive temperature were also normal (our unpublished results). In three different time courses the percentage of cells able to perform the first meiotic division was determined. Data were collected for strains carrying only *top2* or both *top2* and *rec7* at permissive and restrictive temperatures. For comparison, the sporulation efficiencies at restrictive temperature were adjusted by dividing them by the sporulation efficiencies of the parallel cultures at permissive temperature (Table 1).

Figure 3 (facing page). content similar to 0 h but are larger because of chromatin decondensation (see Figure 2D) and show a more open chromatin structure. At 4 h the cells have recondensed again, showing the dense nuclear staining. At 8 h nuclei are visible, which show the typical prophase nuclear morphology (horse tail nuclei). During this time DNA replication takes place. In wild type at 11 h, shortly before the first meiotic division, the nuclei condense again, showing a dense nuclear staining. In the *top2* mutant at 11 h the cells do not condense and show a more open chromatin structure. Bar, 10 μm.

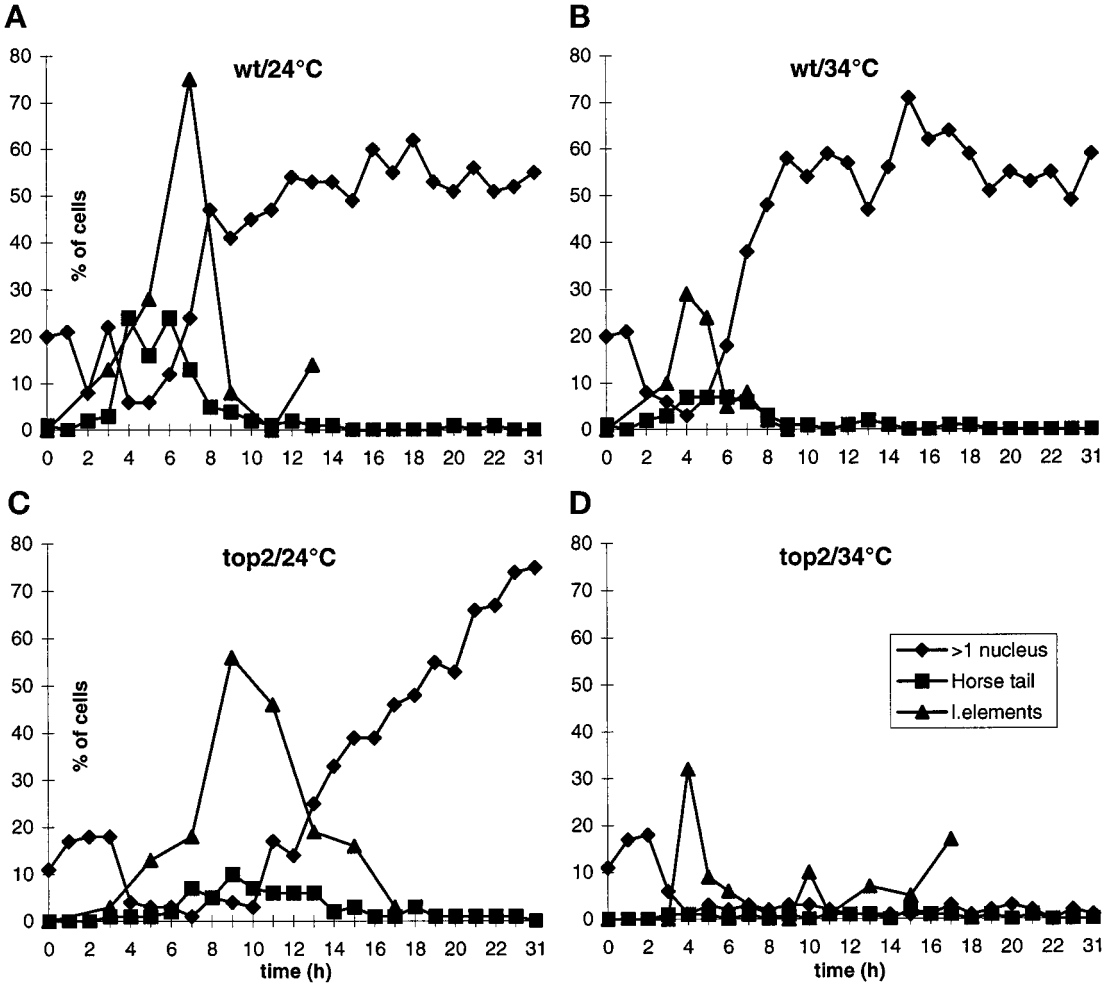


Figure 4. Meiotic time courses with the *top2* mutant and the wild type (wt) at 24°C (permissive temperature) and 34°C (restrictive temperature). For details see MATERIALS AND METHODS. The delay in linear element formation in the *top2* strain at 24°C (C) was within the normal variation between the different time courses. In the wild-type cells at 24°C (A, 13 h) and in the *top2* cells at 34°C (D, 17 h) the percentage of linear element containing cells seemed to increase after a while. This was due to a spreading artifact, caused by selection against cells that were in late stages of meiosis and were not properly protoplasted. This led to an overrepresentation of early cells late in the time courses. In the *top2* strain at 24°C (C) the meiotic divisions were delayed, possibly because the *top2* mutant is not completely wild type at permissive temperature (also see Figure 1 legend). >1 nucleus, percentage of cells that performed the first meiotic division; Horse tail, percentage of cells that show a nuclear morphology typical of prophase after DAPI staining and fluorescence microscopy; l. elements, percentage of cells that contain linear elements (electron microscopy).

In the *top2 rec7* strain the adjusted average value for successful meiosis I was approximately four times higher than in the *top2* strain. Thus we concluded that the *top2* arrest is partly solved in the *top2 rec7* double mutant.

DISCUSSION

We studied meiosis in a heat-sensitive *top2* mutant of *S. pombe*. With a complementary temperature shift experiment we showed that the function of topoisomerase II is not required during meiotic prophase but at the time of the meiotic divisions. DNA replication is normal. Topoisomerase II is necessary for chromatin condensation shortly before meiosis I but not for early decondensation and recondensation. Nuclear morphology and the dynamics of the linear elements during meiotic prophase are normal at restrictive temperature. The cells are blocked at the first meiotic division, but the spindle pole body cycle continues. Finally, we showed that the arrest is partly solved in a *top2 rec7* double mutant.

Interlocks

During the process of meiotic chromosome pairing, chromosomes (or chromosome pairs) can get trapped

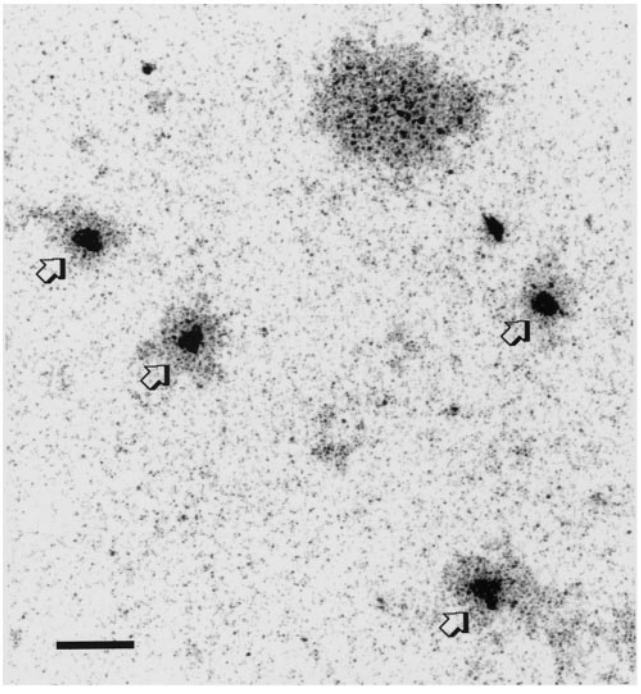


Figure 5. Electron micrograph of a spread preparation of an arrested *top2* cell. Four spindle pole bodies (arrow) are visible in one nucleus. The larger dark-staining area represents the nucleolus. Bar, 1 μm.

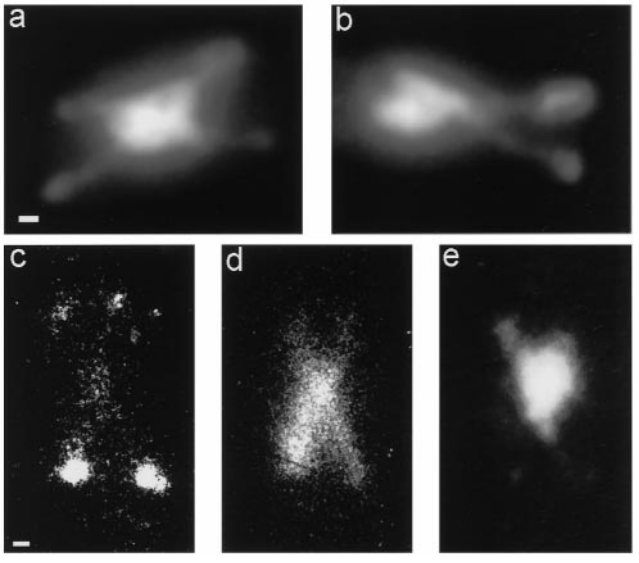


Figure 7. (a and b) Two examples of DAPI-stained, arrested *top2* cells. Chromatin fibers are stretched crosswise out of the nucleus. Bar, 1 μm. (c–e) Immunofluorescence micrographs of an arrested *top2* cell at restrictive temperature. Spindle pole bodies (c) and spindles (d) were stained with specific antibodies (for details see MATERIALS AND METHODS). The nucleus is visualized by DAPI staining (e). Four spindle pole bodies (c) and two crossed meiosis II spindles (d) are visible in a cell that contains only one nucleus, of which DNA fibers are pulled out (e). Bar, 1 μm.

between the partners of a homologous chromosome pair. This is called an interlock. Different mechanisms have been proposed to prevent the formation of interlocks during early prophase. In general, in late pachytene almost all of the interlocks are resolved (von Wettstein *et al.*, 1984; Kleckner and Weiner, 1993). It has been proposed that topoisomerase II might have a function in the resolution by resolving the DNA component of the interlock (Rasmussen, 1986). Rose and Holm (1993) suggested that the regulatory arrest that they observed in a *S. cerevisiae* cs *top2* mutant at restrictive temperature could be triggered by the presence of unresolved interlocks and that the synaptonemal complex could play a role in their detection. In spread preparations of the *S. cerevisiae top2* mutant no interlocks or abnormalities are visible (Rose and Holm, 1993). This suggests that interlocks are not formed at a very high frequency in *S. cerevisiae* and thus are unlikely to be the basis for the complete meiosis I arrest. Assuming that the *rad50* mutation does not prevent the formation of interlocks, the result that the *S. cerevisiae top2 rad50* double mutant is able to perform the first meiotic division (Rose *et al.*, 1990) indicates that in the double mutant interlocks are not preventing chromosome separation in *S. cerevisiae.*

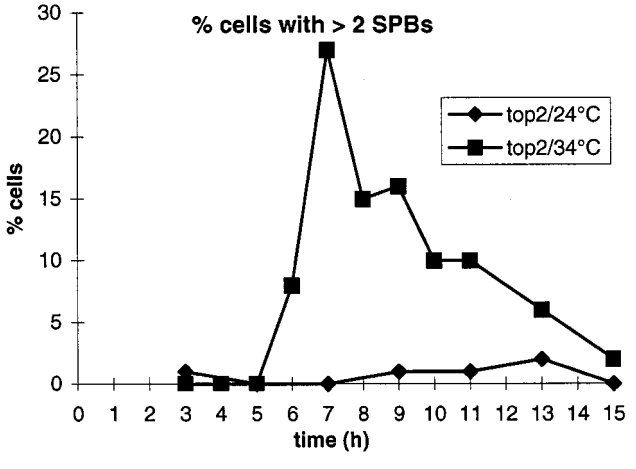


Figure 6. Quantitation of spindle pole bodies. For different time points at permissive and restrictive temperatures, the percentage of *top2* cells containing more than two spindle pole bodies is shown. The data are from the same meiotic time course as presented in Figure 4. For further explanation see RESULTS.

Throughout *S. pombe* meiotic prophase a chromosome bouquet with clustered telomeres and centromeres is maintained (Scherthan *et al.*, 1994), making it unlikely that interlocks are formed at high frequency. It is hardly conceivable that failure of interlock resolution explains the complete nonregulatory arrest at

meiosis I in the *top2* mutant, even when topoisomerase II does resolve interlocks during meiotic prophase.

Untangling of Sister Chromatids and Chromatin Condensation

In mitosis topoisomerase II is necessary for the segregation of sister chromatids after DNA replication (see INTRODUCTION). At the first meiotic division the homologous chromosomes are separated. For separation of homologous chromosomes having one or more crossovers the same task has to be fulfilled as in mitosis: separation of sister chromatids that are entangled because of meiotic DNA replication (Rose *et al.*, 1990). So it is conceivable that the inability of a topoisomerase II mutant to separate sister chromatids leads to arrest at the first meiotic division. This hypothesis is supported by our observation that in a *top2 rec7* mutant the arrest is partly solved. We estimate that the reduction in crossover frequency in the *rec7* mutation is ~40-fold (calculated from data of DeVeaux and Smith, 1994). This means that on average each cell forms approximately one crossover (45 in wild type; Munz, 1994). If we assume that the residual crossovers are distributed according to Poisson, 37% of the meiocytes gets no crossover at all. This is a rough estimate of the fraction of cells that are expected to complete the first meiotic division in the *top2 rec7* double mutant. As shown in Table 1, the observed value is somewhat lower (23%).

In *S. pombe* topoisomerase II is necessary for mitotic chromosome condensation (Uemura *et al.*, 1987). Holm (1994) suggested that chromosome condensation may provide the directionality for disentanglement of sister chromatids by topoisomerase II. Different studies suggest that the processes of chromatid separation and chromatin condensation are linked (reviewed by Yanagida, 1995; Koshland and Strunnikov, 1996). Untangling of sister chromatids possibly takes place in concert with chromatin condensation, and a defect in one of these processes could lead to a defect in the other. If this is true, what could be the primary meiotic defect in the *S. pombe top2* mutant, chromatin condensation or sister chromatid separation? We have shown that early in a meiotic time course, before meiotic DNA replication (see Figure 2, A and D), topoisomerase II is not necessary for decondensation and recondensation. Because these events happen after the mitotic division, at a time when meiotic chromosome pairing begins (Scherthan *et al.*, 1994), we think that these are early meiotic prophase events. The recondensation may be comparable with the leptotene chromosome condensation that marks the beginning of meiotic prophase in other organisms. Whereas topoisomerase II is not needed for early meiotic prophase chromatin condensation, it is required for condensation shortly before meiosis I. This is a remarkable observation and suggests that topoisomerase II is not primarily responsible for chromatin condensation but for the separation of sister chromatids. The defect in decatenating the sister chromatid DNA would then lead to loss of chromatin condensation shortly before meiosis I. This interpretation is supported by the observation that in the *top2* mutant of *S. cerevisiae* no defect in early meiotic chromosome condensation is visible (Rose and Holm, 1993). In this organism chromosome condensation takes place in early prophase, after DNA replication (Padmore *et al.*, 1991; Scherthan *et al.*, 1992). The observation that in *S. pombe* vegetative cells topoisomerase II is not required for prophase condensation (Uemura *et al.*, 1987) is consistent with our results. Thus, although chromosome condensation seems to be necessary for proper segregation (for review, see Yanagida, 1995; Koshland and Strunnikov, 1996), topoisomerase II might not be the enzyme that is directly responsible for chromatin condensation.

Andreassen *et al.* (1997) found that in (nonmeiotic) mammalian cells, override of a checkpoint imposed by VM-26 led to the formation of fully condensed chromosomes, whereas override of an ICRF-193 checkpoint arrest only resulted in partially condensed chromosomes. VM-26 blocks the topoisomerase II strand passing reaction at a step at which the enzyme is covalently bound to a cleaved DNA double strand, whereas ICRF-193 locks the enzyme in a closed clamp formation before DNA cleavage or strand passage. They proposed that in mammalian cells topoisomerase II not only plays an enzymatic role in decatenation but also has a nonenzymatic, structural function, which is necessary for the final steps of chromosome condensation (Andreassen *et al.*, 1997, and references cited therein).

VM-26 causes severe fragmentation of the DNA, ranging from 20 to 800 kb (Roberge *et al.*, 1990). The double-strand breaks caused by VM-26 possibly relieve catenation between the two sister chromatids, permitting the final steps of DNA condensation. In contrast, ICRF-193 does not cause DNA fragmentation (Andreassen *et al*, 1997). Thus the differences between checkpoint-overridden VM-26 and ICRF-193 arrest could alternatively be explained by the different mechanisms in which both drugs act. The partial condensation in the checkpoint-overridden ICRF-193 arrest suggests that catenation prevents complete chromosome condensation.

We suggest that the inability to disentangle sister chromatid DNA is the primary defect in the *top2* mutant causing loss of chromatin condensation and failure of sister chromatid separation.

DNA Catenation and Sister Chromatid Cohesion

In mitosis a system is needed to keep the sister chromatids together until the mitotic division, to ensure proper

Table 1. Partial resolution of the *top2* arrest in the double mutant *top2 rec7*

	Cells performing meiosis I (%)					
	top2			*top2 rec7*		
Exp.	24°C	34°C	34°C adjusted[a]	24°C	34°C	34°C adjusted[a]
1	75	1	1	46	6	13
2	83	11	13	52	22	42
3	70	2	3	53	7	13
Average	76	5	6	50	12	23

[a]The adjusted values were obtained by standardization to the efficiency of meiosis I at 24°C. For further explanation see RESULTS.

segregation (for review, see Holm, 1994). Murray and Szostak (1985) suggested that entangling of the sister chromatid DNA is the primary force that keeps them together until mitotic anaphase. However, in vegetative cells of *S. cerevisiae*, catenation is resolved immediately after S phase (Koshland and Hartwell, 1987; Guacci *et al.*, 1994; for review, see Holm, 1994). From the temperature shift experiment we concluded that in *S. pombe* topoisomerase II is not needed until the time of the meiotic divisions. However, it cannot be excluded that normally in wild type topoisomerase II decatenates the sister chromatid DNA earlier, but that decatenation can be postponed until shortly before the first meiotic division. Molnar *et al.* (1995) have shown with fluorescence in situ hybridization that in a *S. pombe rec8* mutant the sister chromatid cohesion is lost during meiotic prophase. When in *S. pombe* catenation is resolved at the end of meiotic prophase, as suggested by the temperature shift experiment, this means that catenation is not able to prevent loss of sister chromatid cohesion in the *rec8* mutant. Alternatively, catenation is resolved earlier during meiotic prophase (see above). Either way, it suggests that in *S. pombe* meiosis, catenation is not necessary for sister chromatid cohesion.

Nonregulatory Arrest

S. pombe top2 cells arrest before meiosis I. The formation of two spindles and the stretching of chromatin fibers out of the undivided nucleus suggest that the cell tries to perform not only the first but even the second meiotic division. The small percentage of cells containing two spindles at later time points suggests that the spindle formation is transient. Arrested cells contain four spindle pole bodies. Thus the spindle pole body cycle continues despite the blocked nuclear division, indicating that the *top2* arrest is nonregulatory. Also in mitosis the *top2* arrest in *S. cerevisiae* and *S. pombe* is nonregulatory (see INTRODUCTION). Downes *et al* (1994) showed that in mammalian cells a catenation-sensitive checkpoint exists. Rose and Holm (1993) concluded that in *S. cerevisiae* the meiotic *top2* arrest is regulatory and proposed that the synaptonemal complex might play a role in triggering the regulatory arrest. The fact that in *S. pombe*, in which no synaptonemal complex is formed, the meiotic arrest is nonregulatory is consistent with this proposal.

Conclusions

It has already been known for some time that topoisomerase II has a role in sister chromatid separation as well as in chromatin condensation (see INTRODUCTION). But, to our knowledge, a link between these events has not been established. The use of a meiotic system not only enabled us to study the meiotic function of topoisomerase II, but also showed that topoisomerase II is not necessary for chromatin condensation per se, which has implications for the general function of topoisomerase II. Based on our results, we suggest that the primary defect in the *top2* mutant is the inability to disentangle sister chromatid DNA after replication. This leads to failure of sister chromatid separation, a loss of chromatin condensation shortly before meiosis I, and a nonregulatory arrest.

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