

DNA topoisomerase 2 mutant allele mildly delays the mitotic progression and activates the checkpoint protein kinase *Chk1* in fission yeast *Schizosaccharomyces pombe*

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Summary

DNA topoisomerases are specialized nuclear enzymes that perform topological modifications on double-stranded DNA (dsDNA) and hence are essential for DNA metabolism such as replication, transcription, recombination, condensation and segregation. In a genetic screen, we identified a temperature-sensitive mutant allele of topoisomerase 2 that exhibits conditional synthetic lethality with a *chk1* knockout strain. The mutant allele of topoisomerase 2 is defective in chromosome segregation at a non-permissive temperature and there was increase in chromosome segregation defects in the double mutant of *top2-10* and *chk1* delete at a non-permissive temperature. More importantly, topoisomerase 2 mutant cells mildly delay the mitotic progression at non-permissive temperature that is mediated by checkpoint protein kinase *Chk1*. Additionally, *top2-10* mutant cells also activate the *Chk1* at a non-permissive temperature and this activation of *Chk1* takes place at the time of mitosis. Interestingly, *top2-10* mutant cells retain their viability at a non-permissive temperature if the cells are not allowed to enter into mitosis. Taking together our results, we speculate that in the *top2-10* mutant, the segregation of entangled chromatids during mitosis could result in delaying the mitotic progression through the activation of *Chk1* kinase.

1. Introduction

DNA topoisomerases are required for altering DNA topology to facilitate replication, transcription and chromosome segregation. Eukaryotic type II DNA topoisomerases carry out these alterations in DNA structure by transiently introducing breaks in DNA via an ATP-dependent reaction and thus changes the linkage number in DNA molecules (Gellert, 1981; Wang, 1985). This process reversibly decatenates double-stranded DNA (dsDNA) and modulates supercoiling that is essential for mitosis (Yanagida & Wang, 1987). Top2 has been studied in detail in yeast and mammalian systems and has been suggested to be involved in cell proliferation and cell cycle regulation (Smith *et al.*, 2002; Wang, 2002). In the absence of Top2, cells proceed to catastrophic mitosis, indicating the involvement of biochemical surveillance systems or checkpoints in response to the decatenatory activity of Top2 during mitosis (Downes

et al., 1994). However, these checkpoints are not robust enough to maintain cell cycle arrest rather they transiently delay the cell cycle to allow some extra time for the recovery (Gimenez *et al.*, 2002). Conditional mutants of budding and fission yeast lacking *top2* activity at the restrictive temperature cannot form colonies because of the chromosomal breakage generated due to the mis-segregation of sister-chromatids that have not been disentangled during DNA replication (Bermejo *et al.*, 2007). Mammalian G2 cells treated with topoisomerase inhibitors delays entry into mitosis (Kalwinsky *et al.*, 1983; Wan *et al.*, 1999) and drugs that can override checkpoints such as caffeine, bypass these delays (Downes *et al.*, 1994). Thus, it was suggested that this effect was due to a G2 checkpoint that monitors Top2 activity. These inhibitors induced G2 delays without directly causing DNA strand breaks (Tanabe *et al.*, 1991; Downes *et al.*, 1994). Recently, it has been suggested that these inhibitors may induce DNA damage due to interrupted decatenation, indicating an overlap between G2 DNA damage and Top2 checkpoints (Dominguez *et al.*, 2001; Mikhailov *et al.*, 2002; Hajji *et al.*, 2003; Adachi *et al.*, 2004). Studies

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Table 1. *Strains used in this study*

| Strain | Genotype | Source |
|--------|--|----------------|
| SP6 | <i>h⁻ leu1-32</i> | Lab stock |
| NW158 | <i>h⁺ leu1-32 ura4D18 chk1::ura4 ade6-216</i> | Nancy Walworth |
| NW223 | <i>h⁺ leu1-32 chk1.ep ade6-216</i> | Nancy Walworth |
| SH12 | <i>h⁺ leu1-32 top2-10 ade6-210</i> | This study |
| SH3 | <i>h⁺ leu1-32 ura4D18 chk1::ura4 top2-10 ade6-216</i> | This study |
| SH155 | <i>h⁺ leu1-32 ura4D18 top2-10 chk1.ep ade6-216</i> | This study |
| SH165 | <i>h⁻ leu1-32 cdc25-22 chk1.ep ade6-216</i> | This study |
| SH192 | <i>h⁺ leu1-32 cdc25-22 top2-10 chk1.ep ade6-216</i> | This study |

with temperature sensitive *top2* mutants in budding and fission yeast suggest that Top2 plays an important role in decatenating sister chromatids before anaphase (DiNardo *et al.*, 1984; Uemura & Yanagida, 1984; Holm *et al.*, 1985) but their involvement in mitotic checkpoint is still elusive. CPT, a topoisomerase I inhibitor activates *Chk1* and induces a DNA damage checkpoint response in fission yeast (Wan *et al.*, 1999). Recently, it has been proposed that compromised Top2 function activates a checkpoint system that relies on a subset of spindle checkpoint protein rather than DNA damage checkpoint. However, this checkpoint system is operated by a novel mechanism that is independent of the *pds1*/securin pathway (Andrews *et al.*, 2006). In the present study, we have demonstrated a conditional synthetic lethality of a Top2 mutant with DNA damage checkpoint kinase *Chk1*. In the absence of *Chk1*-dependent checkpoint response *top2* mutant cells fail to delay mitotic progression and exhibit chromosome segregation defects. Phosphorylation of the checkpoint protein kinase *Chk1* takes place in the presence of defective *top2* activity during mitosis and this activation of *Chk1* requires cells to pass through the G2 phase of the cell cycle. Furthermore, treating cells with microtubule poison (TBZ) partially increases the survival of *top2-10* mutant cells at a non-permissive temperature.

2. Materials and methods

(i) *Strains and growth condition*

Schizosaccharomyces pombe strains used in this study are listed in Table 1. Standard genetic methods were utilized for making strains as described in Moreno *et al.* (1991). For temperature-shift experiments, cells were grown to mid-log phase at 25 °C and then shifted to restrictive temperature 34 °C in a water bath. To assess checkpoint arrest, cells were synchronized in S phase by growing them in 12 mM hydroxyurea (HU) for 4 h at 25 °C, washed and released at 34 °C. The samples were collected at a 30 min interval and the fraction of binucleate or divided cells was determined as an indication of entry into mitosis using

4',6' diamidino-2-phenylindole (DAPI) staining as described previously (Ahmed *et al.*, 2004).

(ii) *Identification of gene by complementation analysis*

In order to identify the gene coding for *ts10/top2-10* mutant, the *S. pombe* genomic DNA library was transformed in *ts10/top2-10* mutant strain by lithium acetate method (Moreno *et al.*, 1991). *Leu⁺ Ts⁺* transformants were obtained and plasmid was recovered from *Leu⁺ Ts⁺* co-segregants. Restriction digestion and sequencing analysis identify ~15.4 kb region containing two open reading frames. The 4 and 8 kb *HindIII* fragment coding for *apc10* and *top2* genes, respectively, were further sub-cloned in *psp1* vector and re-transformed in *ts10/top2-10* mutant strain for complementation analysis.

(iii) *Microscopy*

For staining nucleus, cells were grown to mid-log phase at 25 °C then shifted to 34 °C, samples were collected at indicated times, fixed with 70% ethanol and stained with DAPI and/or calcofluor, visualized using a fluorescence microscope. About 200–300 cells were counted for aberrant nuclei and percentage was plotted.

(iv) *Preparation of lysates and Western-blot analysis*

Strains with a triple-haemagglutinin tagged allele of *chk1* integrated at the genomic *chk1* locus (*chk1:ep*) were used (Walworth & Bernards, 1996). Cells were grown to mid-log phase at 25 °C then shifted at 34 °C in water bath for different time periods. Cells were harvested by centrifugation and lysed using glass beads and a vortex machine. Lysate in phosphate-buffered saline was centrifuged at 5000 rpm in a microfuge for 5 min at 4 °C. Supernatant was collected and protein estimation was performed using the Bradford assay method. For Western-blot analysis, about 200 µg of total cell lysate was run on 8% SDS-PAGE, transferred to nitrocellulose membrane and probed with F7 (Santa Cruz) or 12CA5 antibody as described earlier (Walworth & Bernards, 1996).

A peroxidase-coupled secondary antibody and the enhanced chemiluminescence detection system (GE lifesciences) were used to detect the immune complexes.

(v) *Cloning of mutant top2 gene by the gap repair method*

Mutant topoisomerase gene was cloned by the gap repair method. Wild-type *top2* gene cloned in psp1 vector was digested with *StuI*–*SphI* restriction enzyme that deleted 1412 amino acids of *top2* coding region. Then 10.7 kb linear fragment containing upstream and downstream sequences of *top2* gene with a leucine selectable marker was isolated by gel purification and transformed in *top2*–10 mutant strain. Transformants containing plasmid with mutant allele of *top2* gene was distinguished from those with integrated plasmid by the mitotic stability of leucine marker present on the plasmid. Plasmid containing mutant copy of *top2* gene was recovered from the transformant as described in Moreno *et al.* (1991), and was confirmed with *HindIII* digestion. The complete gene coding for *top2* was sequenced using appropriate primers and was compared with the wild-type sequence of *top2* gene.

3. Results and discussion

(i) *Identification of mutation leading to synthetic lethality in combination with chk1 deletion*

In order to identify conditional lethal mutants, the *chk1* knockout strain was mutagenized by ethyl methanesulphonate (EMS). Mutant cells that do not form colonies at a non-permissive temperature (36 °C) were subsequently isolated. Thirty-seven such mutants were checked for their inability to grow at the semi-permissive temperature (33 °C). Five such mutants that were unable to form colonies at 33 °C were backcrossed and their ability to grow at 33 °C in wild-type and *chk1* knockout background was compared. One such mutant (*ts10/top2*–10) was able to grow at 33 °C but in *chk1* deletion background the cells were unable to form colonies (Fig. 1(a)), indicating a conditional lethality of *ts10/top2*–10 mutant with *chk1* knockout. Even at the permissive temperature (25 °C) the *ts10/top2*–10 *chk1* deletion strain exhibits slower growth compared to the *ts10/top2*–10 single-mutant strain (Fig. 1(a)), indicating a more severe defect in the double mutant as compared to the single mutant. To further confirm the conditional lethality associated with the *chk1* knockout, we checked the ability of *ts10/top2*–10 and *chk1* delete *ts10/top2*–10 double-mutant strain to form colonies at the permissive temperature after transiently exposing them to a non-permissive temperature (34 °C). The colony-forming ability of *chk1* delete *ts10/top2*–10

double mutant is less as compared with the *ts10/top2*–10 single mutant after exposing the cells at 34 °C for a short period of time (Fig. 1(b)). The inability of the *ts10/top2*–10 mutant to form colonies in the *chk1* knockout background indicates that in the absence of normal checkpoint response the *ts10/top2*–10 mutant cells lose viability at a faster rate compared with the cells exhibiting proficient checkpoint response.

(ii) *Identification of gene coding for ts10/top2–10 by complementation analysis*

The gene coding for the *ts10/top2*–10 mutation was identified by complementation analysis as described in section 2. Sequencing and database analysis identified a 15.4 kb region containing two open reading frames coding for *top2* and *apc10* genes, respectively (Fig. 2(b)). Further sub-cloning and complementation analysis identified *top2* as the *ts10* coding gene, while the *apc10* gene could not complement the temperature-sensitive phenotype of the *ts10/top2*–10 mutant (data not shown).

(iii) *The ts10/top2–10 mutant exhibits chromosome segregation defects at the non-permissive temperature*

To further investigate the defective phenotype in the *top2*–10 mutant, we grew cells at 25 °C and then transferred them to 34 °C for 6 h. DAPI staining was subsequently performed using a fluorescence microscope. In *top2*–10 mutant cells, nuclear chromatin region was not properly segregated but the cells were separated due to the cytokinesis (Fig. 2(a)). About 21% *top2*–10 mutant cells exhibit these chromosome segregation defects after 6 h at the non-permissive temperature compared to wild-type cells that did not exhibit segregation defects under the same growth conditions (Fig. 2(a)). Additionally, a number of cells in *top2*–10 mutant were having single nuclei at the edge of the cells (Fig. 2(a), dotted arrow). These nuclei could have been generated due to the separation of two daughter cells having aberrant mitosis. Consistently different mutant alleles of *top2* have also been shown to exhibit similar kinds of chromosome segregation defects (Uemura & Yanagida, 1984, 1986).

(iv) *Combination of top2–10 with the chk1 deletion aggravates the segregation defects*

The lethality of the *top2* mutant in *chk1* deletion background at a semi-non-permissive temperature prompted us to look for mitotic defects in the *chk1* knockout and *top2*–10 double-mutant strains. Wild-type, *chk1* delete, *top2*–10 mutant and *top2*–10 *chk1* delete strains were grown at permissive temperature (25 °C) till the mid-log phase and then shifted to a non-permissive temperature (34 °C). Samples were taken at regular time intervals. Wild-type and *chk1*

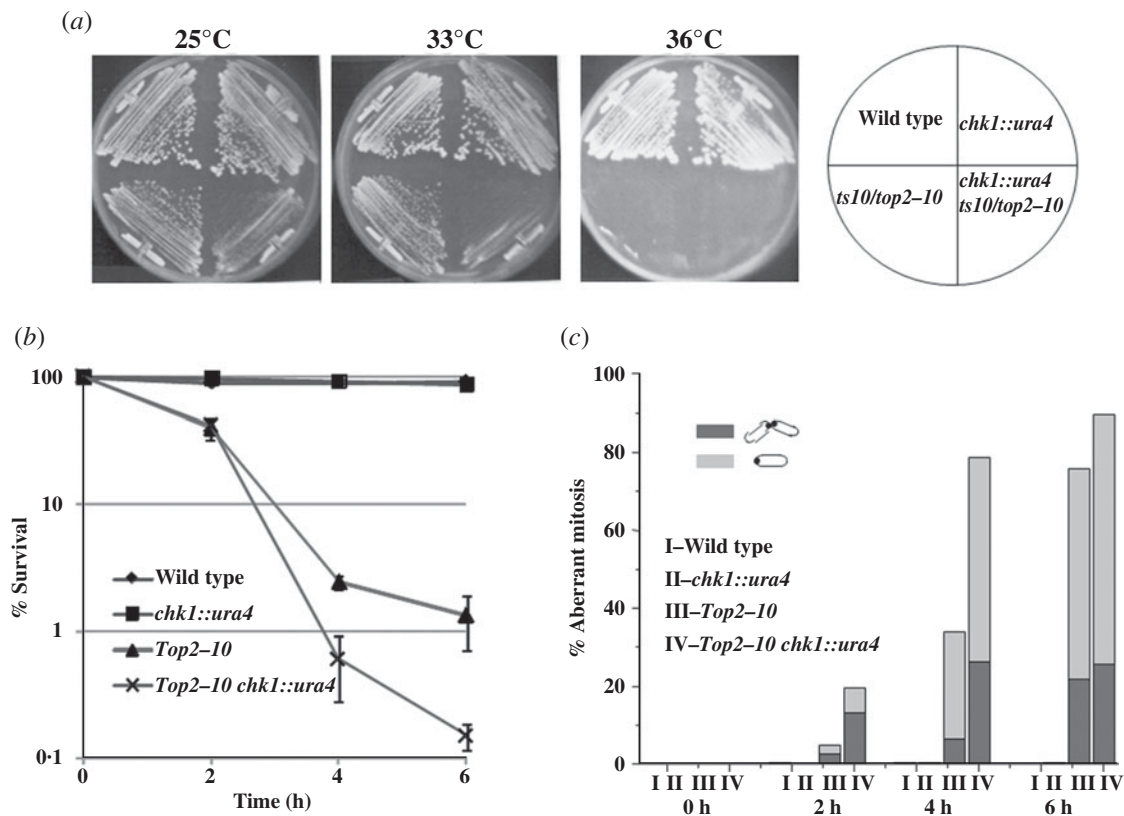


Fig. 1. The *ts10/top2-10* mutant allele shows conditional lethality with *chk1* knockout: (a) Indicated strains were streaked on YEA plates and incubated at indicated temperature for 2–4 days before taking photograph. (b) Per cent survival in *top2-10 chk1* knockout strain at a non-permissive temperature: indicated strains were grown till mid-log phase at 25 °C then shifted at 34 °C. Sample were collected at 2 h intervals, equal number of cells were plated on YEA plates and incubated at 25 °C. Number of surviving colonies was calculated as the percentage of colonies appearing at permissive temperature. Values shown are the average of three independent experiments with standard deviation. (c) Per cent aberrant mitosis in *top2-10* mutant cells at non-permissive temperature: indicated strains were grown till mid-log phase at 25 °C and then shifted at 34 °C. Cells were collected at the indicated time interval, fixed with 70% ethanol and stained with DAPI. Cells having aberrant chromosome segregation were counted.

delete cells exhibit normal chromosome segregation, while about 78% *top2-10 chk1* delete cells exhibit chromosome segregation defects during mitosis as compared with *top2-10* single mutant that has only about 34% chromosome segregation defects after 4 h at 34 °C (Fig. 1(c)). During the same period of time there was considerable reduction in survival pattern of *top2-10* and *chk1* delete *top2-10* cells (Fig. 1(b)). Further incubation at non-permissive temperature results in about 75% aberrant mitosis in *top2-10* mutant as compared to 89% in double mutant. These results strengthen our observation that in the absence of a *Chk1*-dependent checkpoint response, *top2-10* mutant cells exhibit increase in chromosome segregation defect.

(v) Mapping and identification of *top2-10* mutation by gap repair

Furthermore, to identify the mutation in *ts10/top2-10* gene we cloned the mutant *top2* gene by gap repair as described in section 2. Transformation of plasmid

containing mutant copy of *top2* gene in *top2-10* mutant strain does not suppress the temperature sensitive phenotype of the mutant strain (data not shown), suggesting that the isolated plasmid indeed contain mutant copy of *top2* gene. Sequencing and comparison with wild-type sequence of *top2* gene indicated a mutation from C to T, which changes Arg 288 to Cys (Fig. 2(c)). The arginine residue at 288 is conserved across the species (Fig. 2(c)) indicating that this residue might be having some important role in topoisomerase function. Furthermore, these results also rule out the possibility that *top2* could be a suppressor of *ts10* mutant strain.

(vi) Topoisomerase 2 mutant cells mildly delay the mitotic progression at a non-permissive temperature that required the checkpoint kinase protein Chk1

In order to investigate the role of *top2-10* mutant in *Chk1*-dependent checkpoint function, we examine mitotic entry at non-permissive temperature in *top2-10* mutant cells. The *top2-10* mutant cells delay

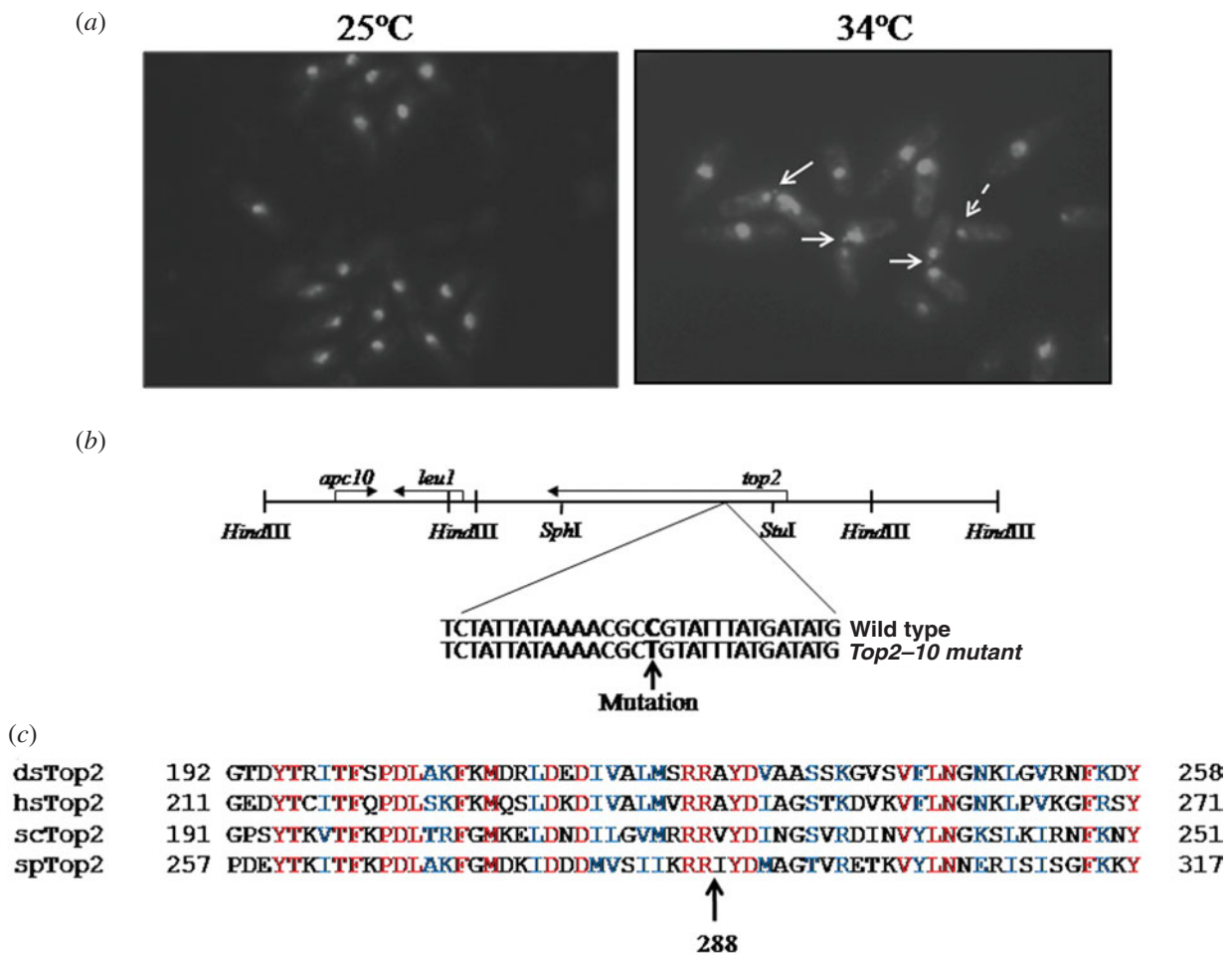


Fig. 2. (a) *top2-10* mutant cells exhibit chromosome segregation defect at a non-permissive temperature: cells were grown at 25 °C till mid-log phase and then shifted at 34 °C for 4 h, fixed with 70% ethanol and stained with DAPI. Arrow indicates defective chromosome segregation. (b) Schematic map of 15.4 kb region of plasmid (upper panel), indicating 8.0 and 4.0 kb *HindIII* fragment containing *top2*⁺ and *apc10*⁺ genes, respectively. Location of mutation in *top2* gene has been shown in the lower panel. (c) Multiple alignment of Top2 from *S. pombe* (spTop2; accession no. P08096), *Drosophila* (dmTop2; accession no. P15348), *S. cerevisiae* (scTop2; accession no. P06786) and humans (hsTop2; accession no. P11388) are shown. The conserved arginine residue at position 288 in *S. pombe* is marked.

mitotic entry at a non-permissive temperature (Fig. 3(a)). More importantly, *top2-10* mutant cells fail to delay mitotic entry in a *chk1* deletion background (Fig. 3(a)), suggesting that the genotoxic stress response in *top2-10* mutant cells at a non-permissive temperature generate a *Chk1*-dependent checkpoint response.

(vii) *Topoisomerase 2 mutant cells activate the DNA damage checkpoint protein kinase Chk1 during mitosis*

In response to DNA damaging agents, the *Chk1* protein gets phosphorylated due to the activation of checkpoint and hence migrates slowly on SDS-PAGE (Walworth & Bernards, 1996). Reduced viability in *top2-10 chk1* deletion background and delay in mitotic progression in *top2-10* mutant cells prompted us to question whether the *top2-10* mutant allele also activates *Chk1*. To ascertain the activation of *Chk1*,

we introduced the HA-tagged *Chk1* in the *top2-10* mutant background by genetic crosses. *Chk1* was monitored by Western blotting probed with anti-HA antibody (F7). As shown in Fig. 3(b), the slow migrating form of *Chk1* starts appearing in *top2-10* mutant cells after 3 h and becomes more prominent after 6 h at a non-permissive temperature (34 °C) indicating phosphorylation of *Chk1* in *top2-10* mutant cells, while the wild-type cells grown under same conditions do not exhibit this phenomenon (Fig. 3(b), upper panel). This result clearly suggests that defects in Top2 activity leads to the activation of the checkpoint kinase protein *Chk1*.

(viii) *Activation of the Chk1 kinase in topoisomerase mutant is a post-G2 event*

The protein kinase *Chk1* is phosphorylated in response to a variety of DNA-damaging agents, such as UV light, topoisomerase I poison camptothecin

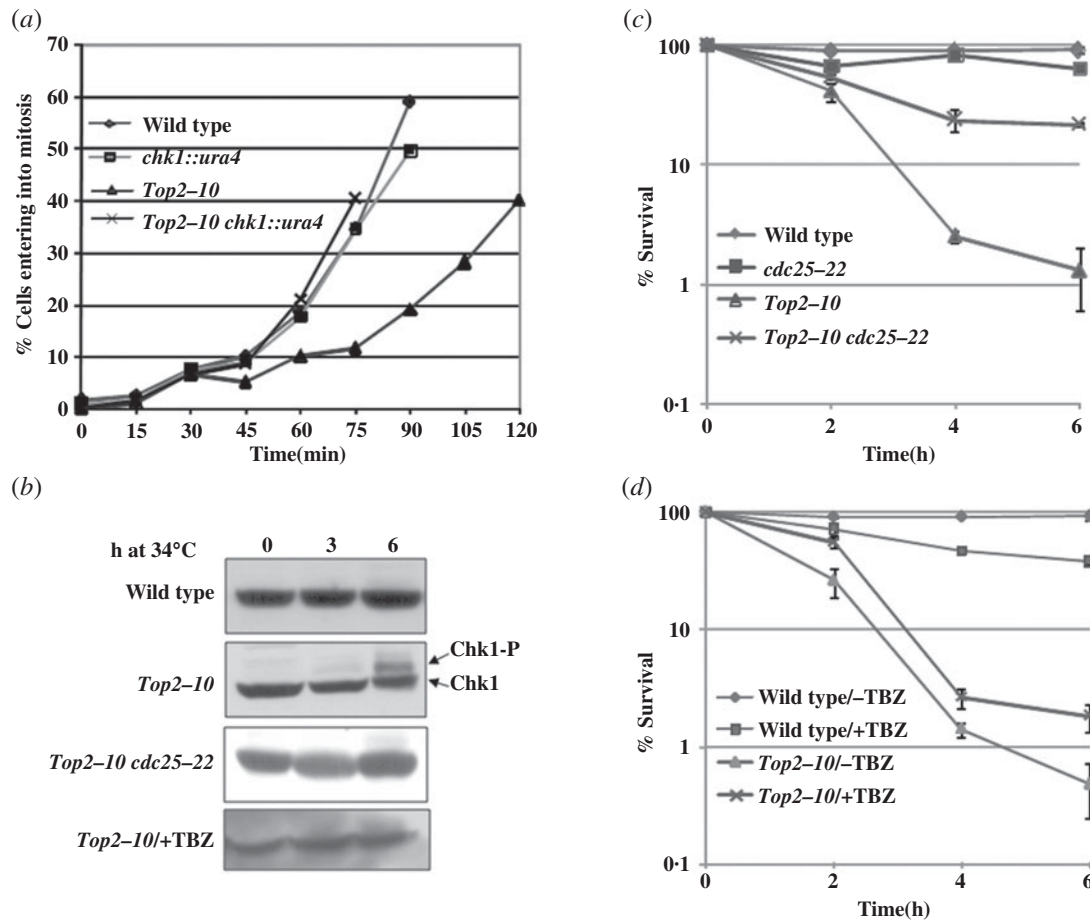


Fig. 3. Topoisomerase 2 mutant cells mildly delay the mitotic progression and activates checkpoint kinase *Chk1*: (a) cells were synchronized in S phase by growing them in 12 mM HU for 4 h at 25 °C, washed and released at 34 °C, samples were collected at 15 min interval and the fraction of binucleate or divided cells was determined as an indication of entry into mitosis using DAPI staining. (b) Indicated strains were grown at permissive temperature till mid-log phase then shifted at 34 °C for indicated time. Protein lysate was prepared as described in *Materials and methods*, samples were run on 8% SDS-PAGE, transferred on nitrocellulose membrane and probed with anti-HA antibody. (c) Per cent survival in *top2-10* mutant cells arrested in G2: indicated strains were grown till the mid-log phase at 25 °C then shifted at 34 °C. Samples were collected at a 2 h interval; equal number of cells were plated on YEA plates and incubated at 25 °C. Number of surviving colonies was calculated as the percentage of colonies appearing at permissive temperature. Values shown are the average of three independent experiments with standard deviation. (d) Per cent survival in *top2-10* mutant cells grown in the presence of TBZ: indicated strains were processed as described in (c) except that the cultures were shifted at 34 °C in the presence or absence of 30 µg/ml TBZ.

(CPT) and inactivation of DNA ligase (Walworth & Bernards, 1996; Wan *et al.*, 1999). A DNA damage signal could be generated in cells harbouring mutation in *top2* gene by one of at least two mechanisms. In one scenario, the double-strand break can be generated in response to defective topoisomerase 2 functions at the time of DNA replication. Alternatively, in *top2* mutants the attempt to segregate entangled chromatids could cause chromosomal breakage at the time of mitosis; a process that might also activate the *Chk1* kinase. To check these possibilities a conditional mutant *cdc25-22* that arrests cell cycle in G2 was used. As shown in Fig. 3(b), *top2-10* mutant cells that were arrested in G2 failed to activate *Chk1* when shifted to a non-permissive temperature (34 °C). This suggests that in *top2* mutants the activation of *Chk1*

takes place only if cells try to enter into mitosis. Furthermore, the survival pattern of the *cdc25-22 top2-10* double mutant was better as compared to the *top2-10* single mutant at 34 °C (Fig. 3(c)). Consistent with this the *cdc25-22 top2-10* double-mutant cells were as long as *cdc25-22* single mutants with single nuclei (data not shown), suggesting an arrest in G2 phase of the cell cycle. Furthermore, there was partial improvement of survival at 34 °C in the *top2-10* mutant cells grown in the presence of microtubule poison (TBZ) as compared with the cells grown in its absence (Fig. 3(d)), suggesting that decrease in viability in the *top2-10* mutant cells could be due to the catastrophic mitosis. There was reduction in viability in wild-type cells treated with TBZ that could be due to some toxic effect of the drug (Fig. 3(d)). This could explain why

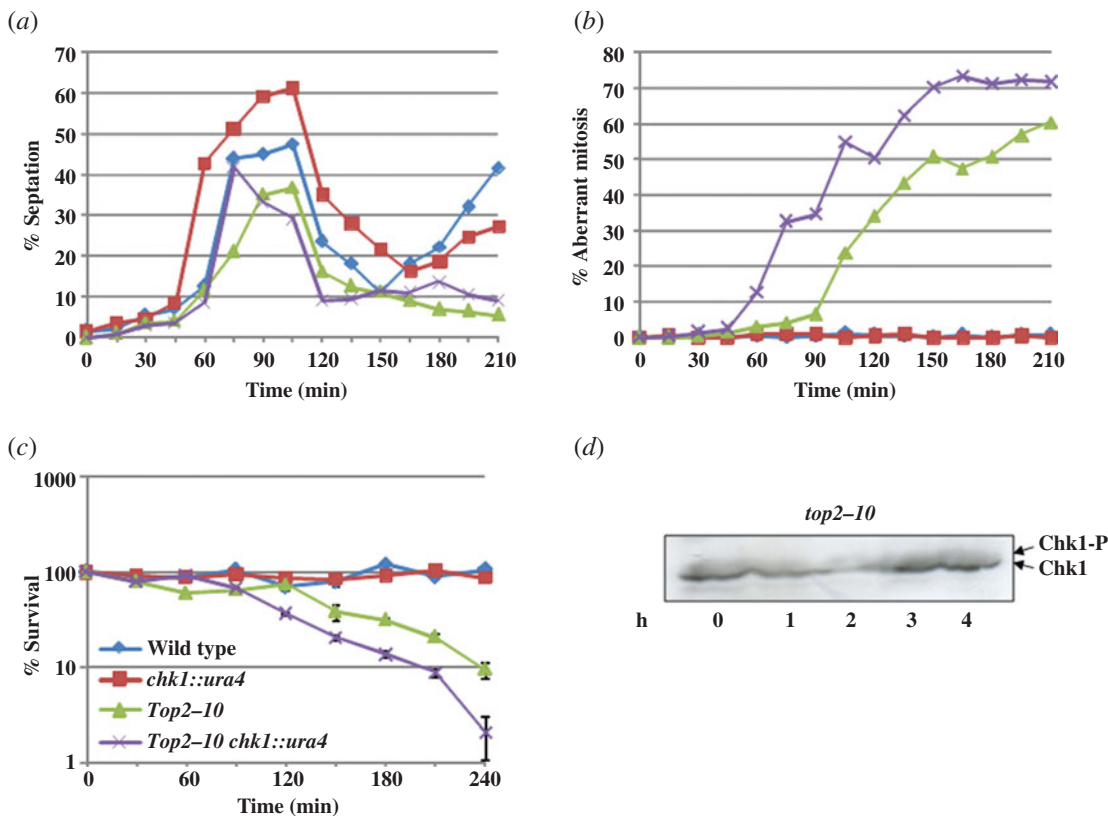


Fig. 4. Cells were synchronized in S phase and released at non-permissive temperature as described in section 2. Samples were collected at 15 min interval, fixed with 70% ethanol and stained with calcofluor or with DAPI. About 200 cells were counted for each sample, (a) percent septation and (b) percent aberrant mitosis was plotted. (c) Per cent survival in synchronized *top2-10* mutant cells: indicated strains were processed as above, samples were collected at a 30 min interval; equal number of cells were plated on YEA plates and incubated at 25 °C. The number of surviving colonies was calculated as the percentage of colonies appearing at permissive temperature. Values shown are the average of three independent experiments with standard deviation. (d) *Chk1* phosphorylation in synchronized *top2-10* mutant cells: cells were synchronized in S phase with 12 mM HU and released at non-permissive temperature as described in section 2. Samples were collected at 1 h time interval. Protein lysate was prepared and Western blot was performed using anti-HA antibody.

there was only marginal improvement of survival in *top2-10* mutant in the presence of TBZ as compared to a robust increase in survival in *cdc25-22 top2-10* mutant cells (compare Fig. 3(d) with Fig. 3(c)).

(ix) Analysis of cell cycle kinetics in synchronized *top2-10* mutant

To monitor the cell cycle progression, we synchronized the cells in S phase by growing them in the presence of 12 mM HU at a permissive temperature for 4 h; the cells were washed and released at a non-permissive temperature (34 °C). During the first cell cycle, the septation index was high at 90 min time points indicating a normal cytokinesis. More importantly, in wild-type and *chk1* delete cells, the septation index starts increasing after 165 min indicating normal second cell cycle progression. In contrast to this, the septation index remained low in *top2-10* and *top2-10 chk1* delete cells, suggesting that these cells were unable to enter into the next cell cycle (Fig. 4(a)),

possibly due to the catastrophic defects just after the first mitosis. There was 30–45 min delay in mitotic entry at a non-permissive temperature in *top2-10* mutant as compared to the *top2-10 chk1* delete cells (Fig. 3(a)). Consistently, only 4% of *top2-10* cells had aberrant mitosis after 75 min shift at a non-permissive temperature as compared to 32% in *top2-10 chk1* delete cells (Fig. 4(b)), indicating the involvement of *Chk1*-dependent checkpoint response in *top2-10* mutant cells at the time of mitosis. Additionally, the per cent survival of *top2-10* mutant and *top2-10 chk1* delete mutant cells decreases as the percentage of aberrant mitosis increases (Fig. 4(c)). To determine the timing of the activation of *Chk1* in *top2-10* mutant cells, the *Chk1* phosphorylation was monitored in synchronized population after releasing from HU arrest. As shown in Fig. 4(d), a small fraction of *Chk1* was phosphorylated in HU-arrested cells in *top2-10* mutant cells. The level of phosphorylation in *top2-10* mutant cells decreases in 1 h coincidentally the percentage of aberrant mitosis was also negligible at the

same time point (Fig. 4(d)). The fraction of phosphorylated form of *Chk1* was marginally increased in the *top2-10* mutant cells after 2 h at a non-permissive temperature (Fig. 4(d)) with high percentage of aberrant mitosis at the same time point (Fig. 4(b)). This result further suggests that the aberrant chromatin structure generated at the time of mitosis could result in phosphorylation of *Chk1* kinase protein although this activation of checkpoint response is not sufficient for the survival of *top2-10* mutant cells.

The absence of checkpoint function in the *top2* mutant results in genome damage and genomic instability. A temperature-sensitive *top2-10* mutant allele exhibits chromosome segregation defects. The double mutants of *top2-10* and *chk1* deletion lose their viability faster than the single *top2-10* mutant at a non-permissive temperature with high percentage of aberrant mitosis as compared to single mutant suggesting that abrogating *top2* function might be activating a checkpoint response involving *Chk1*. Since the *top2-10* single-mutant cells lose their viability with the time suggest that the checkpoint response generated may not be sufficient for cell cycle delay. More recently, in *Saccharomyces cerevisiae* it has been shown that *top1* and *top2* localize at a chromosomal region undergoing replication and are required for solving the topological constraints at the moving replication fork and *top2* mutant cells at a non-permissive temperature activate DNA damage checkpoint response (Bermejo *et al.*, 2007). It can be argued that due to the defective decatenating activity of *top2* in fission yeast, topological constraints at the moving replication fork can be generated that can lead to DNA/chromatin damage at the time of mitosis and hence could activate a DNA damage checkpoint pathway. It is also possible that the aberrant chromosomal structure might be generated due to the entangled chromatid in *top2-10* mutant cells at the time of mitosis and these defects are responsible for the activation of protein kinase *Chk1*. Furthermore, blocking the *top2-10* mutant cells in G2 or exposing them with microtubule poison (TBZ) partially increases their survival at a non-permissive temperature suggest that loss in viability in *top2-10* mutant cells could be due to the catastrophic defects, generated at the time of mitosis. In a previous study by Uemura & Yanagida (1986), it has been shown that the viability of *top2* mutant cells remained high during the G2 phase but decreased sharply at the time of mitosis. We speculate that the decrease in the viability in *top2-10* mutant could be due to the catastrophic segregation of entangled chromatids. The aberrant chromosomal structure generated during mitosis in *top2-10* mutant cells might generate signals that activate the protein kinase *Chk1*. Involvement of *Chk1* pathway to delay metaphase to anaphase transition in the cells harbouring abnormal DNA structure has been shown in

S. pombe and *Drosophila* (Collura *et al.*, 2005; Royou *et al.*, 2005). Further work is expected to unravel the mechanism in the *top2* mutant in detail.

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