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Fission Yeast Topoisomerase II (Top2) is Required for the Viability of Pot1Δ Single Mutant

Research Article

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Abstract

Topoisomerase II constitutes a group of nuclear enzymes which alters the topological state of DNA. These enzymes have the ability to transferring one DNA double helix through a transient break in another DNA double helix. Topoisomerases type II have important roles in DNA metabolic processes such as DNA replication, transcription and chromosome segregation, condensation and de-condensation. Recently Topoisomerases II become very important target for many anticancer drugs such as Doxorubicin, Etoposide, and Aminoacridines. So, our results suggest that Topoisomerase II is required for the viability of pot1Δ cells by creation fission yeast (Shizosaccharomyces Pombe) double mutant model (pot1Δ top2Δ). In addition to the deletion of Rav1 gene enhance the sensitivity of cells to drugs. This leads to developing assay system to screening Topoisomerase II inhibitors by creation (S. Pombe) double mutant model pot1Δ rav1Δ.

Keywords: DNA; Doxorubicin; Schizosaccharomyces Pombe; pot1; top2; rav1.

Introduction

Schizosaccharomyces Pombe is a single celled free living archiascomycete fungus sharing many features with cells is more complicated eukaryotes. From gene sequence comparisons and phylogenetic analyses it has been suggested that fission yeast diverged from budding yeast around 330-420 million years ago, and from metazoan and plants around 1,000-1,200 million years ago [1]. Some gene sequences are as equally diverged between the two yeasts as they are from their human homologues, probably reflecting a more rapid evolution within fungal lineages than in the metazoan *S. pombe* was first described in the 1890s and has been extensively studied since the 1950s [2,3]. It has served as an excellent model organism for the study of cell cycle control, mitosis [4], DNA repair and recombination [5] and checkpoint controls important for genome stability [6]. The 13.8 Mb genome of *S. pombe* is distributed between chromosomes I (5.7 Mb), II (4.6 Mb) and III (3.5 Mb) [7], together with a 20 Kb mitochondrial genome [8]. Topoisomerase II is located at chromosome II (1.976 Mb 1.980 Mb).

DNA Topoisomerases type 2 is essential enzymes to all living cells. These enzymes have the ability to transferring one DNA double helix through a transient break in another DNA double helix [8], while Topoisomerases type 1 makes single strand breaks through the nick to allow relaxation of over-coiled DNA [9]. In eukaryotes the enzymes catalyzes the ATP- dependent relaxation of negatively and positively supercoiled DNA as well as catenation-decatenation and knotting-unknotting circular DNA [10,11] and have important roles in DNA replication, transcription and chromosome segregation, chromosome condensation and decondensation. *Top2* gene is also very important target for a widely number of anticancer drugs in clinical use, such as the anthracyclines [12], which acts as inhibitor for the DNA topology

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regulating enzyme *top2*. Inhibiting of *top2* results in accumulation of DSB that leads to cell death [13]. Furthermore, Topoisomerase poisons can be categorized into three groups; intercalators (such as doxorubicin and m-AMSA), non-DNA-intercalators (etoposide, teniposide) and catalytic inhibitors of the topoisomerase (aclarubicin and bisdioxopiperidines like ICRF-193) [14]. The first two groups trap *top2* on the DNA in a covalently bound state, often referred to as the cleavable complex [15]. Catalytic inhibitors act on *top2* without forming the cleavable complex. It is not entirely clear what causes cell death following topoisomerase stabilization. DNA damage provoked by *top2* poisons was shown in some cases to result in apoptosis [16].

Mechanism of drug resistance

Drug resistant topoisomerase 2 restricts the therapeutic choices in many cases. It is sometimes caused by the overexpression of membrane P-glycoprotein (*MDRl* gene product) which responsible of efflux for many drugs from cells [17]. Others are produced by the *MDRl* independent mechanisms, which in many cases are correlated with alterations of *top2* gene, like the expression level or mutation. The expression level of *top2* is affected by several factors, especially, down regulation of the *top2* mRNA [18], instability of mRNA [19] and destruction of the *top2* protein [20].

Identification of *top2* inhibitors resistance genes in fission yeast

Protection of telomeres protein 1 (*pot1*) binds to single- stranded telomere overhangs and protects chromosome ends [21]. In *S. pombe* the deletion of *pot1* causes rapid telomere loss and chromosome circularization [22] by single strand annealing (SSA), suggesting that the chromosome ends are resected in the absence of *pot1* [23,24] gene.

Rav1 gene encodes the homologue of a budding yeast protein which regulates the assembly of vacuolar ATPase this gene have important role in Multi drug resistance (MDR) [25,26]. The mutation in this gene (*rav1***Δ**) make the cell very sensitive to the chemotherapeutic agents .We found that *rav1Δ* mutant leads to increase the accumulation of the drug in cells [27]. The function of *Rav1* appears to be conserved, *S. Pombe rav1* is part of a RAVE-like complex in fission yeast and loss of *rav1* gene results in an increased sensitivity of S. pombe cells to drugs [28].

The aim of our work was investigate that *top2* gene is required for the viability of *pot1***Δ** cells using *S. Pombe pot1***Δ** *top2***Δ** double mutant and developing assay system to screening *top2* inhibitors using *S. Pombe* double mutant *pot1***Δ** *rav1***Δ** as a sensitive model to drugs.

Materials and Methods

Strain construction and growth media

Fission yeast 972 (h-) strain, YEA (3% glucose, 0.5% yeast extract, and 75 mg/L adenine) media was used to grow fission yeast cells. *pot1***Δ** *rav1***Δ** double mutant (*pot1::KanMX rav1::hphMX*) expressing *pot1* from a plasmid (p^{p}c27-pot1-hemaagglutinin [HA]) was created by genetic interaction (mating cells) between *pot1::kanMX* **Δ** (h^{+}) single mutant which have pot1 plasmid with *rav1::hphMX* **Δ** single mutant (h^{-}) using ME medium (minimal medium Bacto-malt extract 30 g/L) adjusting PH 5.5. The double mutant were selected on Kan plate (YEA containing Kanamycin 1 µL/ mL) , hph plate (YEA containing Hygromycin 1 µL/ mL) and yeast extract agar (YEA) plates containing 2 g/ L 5-Fluoroorotic acid (FOA) and EMM3+ALH plate (Edinburgh minimal media containing alanine, leucine and histidine). The loss of telomeric repeats and chromosome circularization were confirmed by southern hybridization and pulsed-field gel electrophoresis (PFGE) respectively.

*pot1***Δ** *top2***Δ** double mutant (*pot1::KanMX top2:: ura4*) expressing Pot1 from a plasmid (p^{p}c27-pot1-hemaagglutinin [HA], containing the ura4 gene) was created by genetic interaction (mating cells) between *pot1::KanMX* **Δ** (h^{+}) single mutant which have pot1 plasmid with *top2::ura4***Δ** (h^{-}) single mutant using ME medium, the double mutant were selected on yeast extract agar (YEA) plates containing 2 g/ L 5-Fluoroorotic acid (FOA).

Chemicals and solvents

Kanamycin (sigma), hygromycin (sigma), yeast extract (Becton, Dickinson. Company), AlKphos direct kit module (GE Healthcare), EcoRI restriction enzyme and NotI enzyme (Bio labs. Inc.), other chemicals described later.

Measurement of Telomere Length telomere length was measured using southern hybridization with an AlKPhos Direct Kit module (GE Healthcare), according to a previously described procedure [29].

Pulsed-Field Gel Electrophoresis (PFGE) was performed as described by Baumann et al. [30] for The detection of Not I-digested chromosomes, Not I-digested *S.pombe* chromosomal DNA was fractionated in a 1% agarose gel with 0.5×TBE (50 mM Tris-HCl, 5 mM boric acid and 1 mM EDTA, pH 8.0) buffer at 14 °C using the CHEF Mapper PFGE system at 6 v/m (200 v) and a pulse time of 60 to 120 s for 24 h. DNA was visualized by staining with ethidium bromide (1µg/ ml) for 30 min.

Western blot analysis Cells were lysed in 10% trichloroacetic acid (TCA) with glass beads at 4 °C. After centrifugation at 15,000 × g for 10 min at 4 °C, the precipitate was washed with acetone and suspended in SDS sample buffer.

Microscopy Microscope images of living cells were obtained using an AxioCam digital camera (Zeiss) connected to an Axio observer Z1 micro-scope (Zeiss).

DAPI stained a 10×DAPI mounting stock prepared and aliquoted. This is 10 µg/ml DAPI, 10 mg/ml p-phenyl enediamine. (The DAPI stock is 1 mg/ml in DMSO) take a 20 µl aliquot and add 180 µl glycerol this gives a 1×working stock (1 µg/ ml DAPI, 1 mg/ ml p-phenylenediamine which acts as anti-fade, 90% glycerol) which should be kept at -20 °C in the dark.

Cell counting with a Hemocytometer 10 µl cell culture in YEA liquid medium and the cell visualized by normal microscope after 4, 16, 32, 64, 128 hours.

Flow cytometry

Cell Staining With PI

Suspend approx 10^{6}cells in 0.5 mL of PBS. Vortex gently (approx 5 s) to obtain a mono-dispersed cell suspension, with minimal cell

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aggregation ,then fix cells by transferring this suspension with a Pasteur pipet into centrifuge tubes containing 4.5 mL of 70% ethanol on ice. Keep cells in ethanol for at least 2 h at 4 °C, after that Centrifuge the ethanol-suspended cells for 5 min at 300 g, Suspend the cell pellet in 5 mL of PBS wait approx 30 s and centrifuge at 300 g for 5 min, then Suspend the cell pellet in 1 mL of PI staining solution. Keep in the dark at room temperature for 30 min, or at 37 °C for 10 min, finally Transfer sample to the flow cytometer and measure cell fluorescence maximum excitation of PI bound to DNA is at 536 nm, and emission is at 617 nm blue (488nm).

Fluorescence Activated Cell Sorting (FACS) analysis using desktop flow cytometer the Becton-Dickinson fluorescence activated cell sorter.

Spotting assay for growth

Strains were tested by growing to exponential phase followed by spotting individual ten-fold serially diluted strains manually on agar media containing doxorubicin (DOXO), hydroxyl urea (HU), thiabendazole (TBZ) and methyl methane sulphonate (MMS) at different concentrations and incubated at 30 °C for three days or four days as follow, grow strains overnight in 5 ml liquid medium (e.g. YEA) at 30 °C, then Measure OD600and prepare dilutions of OD600= 1 with sterile H2O, dilution series: 100, 10^{-1}, 10^{-2}, 10^{-3} (use spotting plate), then drop dilutions onto appropriate plates using a sterile metal stamper, creating a grid of descending cell concentrations. Let plates dry completely, Incubate at 30 °C. Finally, take photographs at different days of incubation since differences can change quite drastically during growth.

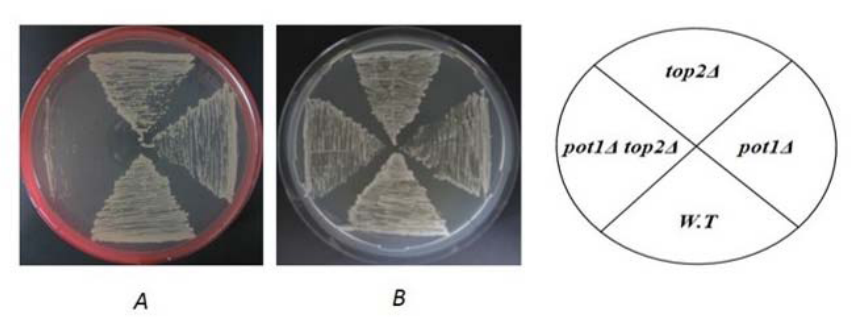

Figure 1: The *pot1***Δ** *top2***Δ** double mutants, wild type, *top2***Δ** single mutant and *pot1***Δ** single mutant (-Pot1p) were plated on YEA. Incubated at different temperatures 25°C (B), 30°C (A). *pot1***Δ** *top2***Δ** double mutant was not viable at 30°C as shown (A).

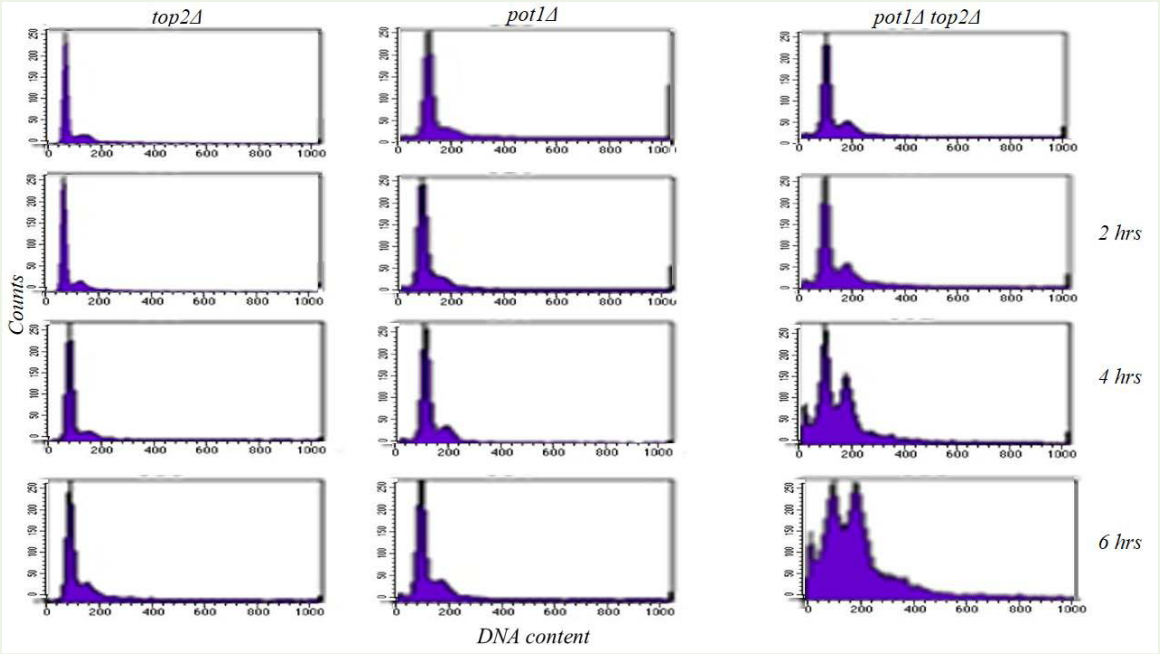

Figure 2: Flow cytometric analysis of DNA content of *pot1***Δ** *top2***Δ** double mutant, *pot1***Δ** single mutant, and *top2***Δ** single mutant after incubation at 25°C overnight. Samples were fixed in ethanol and later stained with either propidium iodide. Linear fluorescence histograms show relative DNA content on the horizontal axis and the cell counts on the vertical axis. Flow cytometric analysis was performed onthe software system (Beckton Dickinson). Results suggested that as showed by comparing the DNA amount between *pot1***Δ** single mutant and *top2***Δ** single mutant and *pot1***Δ** *top2***Δ** double mutant this confirmed the disorders in DNA synthesis during S phase.

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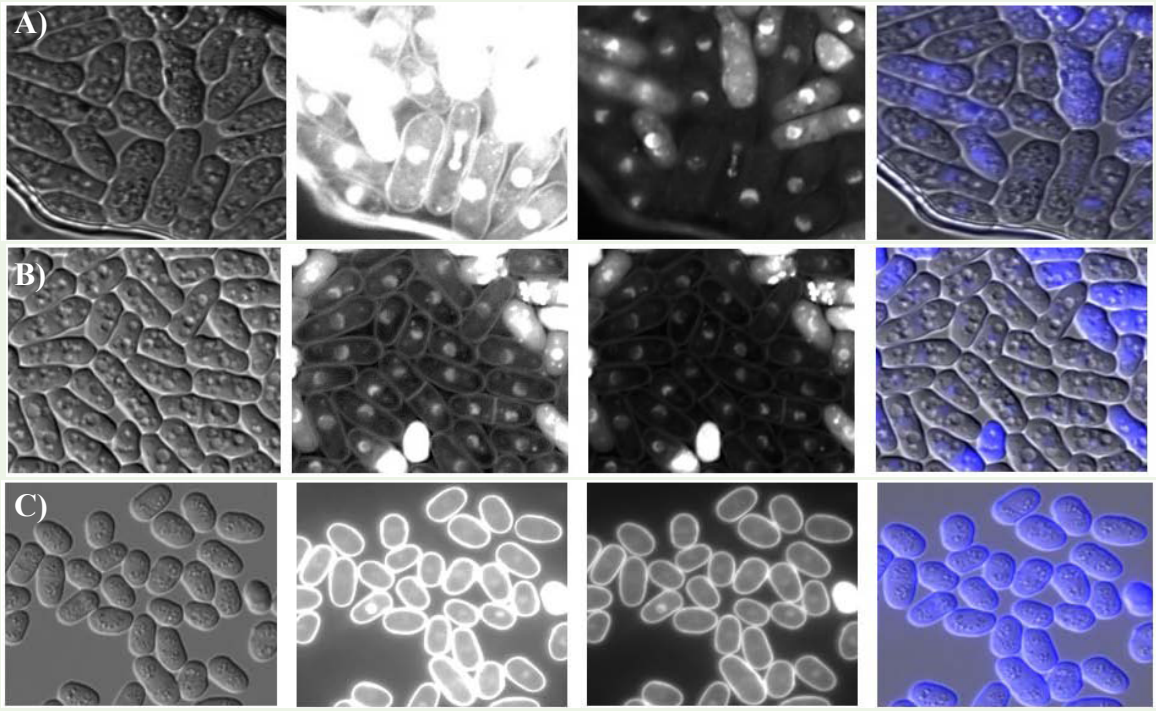

Figure 3: DAPI stained result showed most of *pot1***Δ** *top2***Δ** **(**A**)** double mutant cells is not viable and some of the viable cells have chromosome segregation disorders as shown. The phenotype of *pot1***Δ** *top2***Δ**, *top2***Δ** (C)**,***pot1***Δ** **(**B**)** to detect the difference of cell length. The double mutant have elongated cells compare to *top2* **Δ**, *pot1* **Δ**, this suggest that DNA checkpoint activation because DNA damage during chromosome segregation and DNA replication. Cell shape results showed most of *pot1***Δ** *top2***Δ** cells have elongated cells.

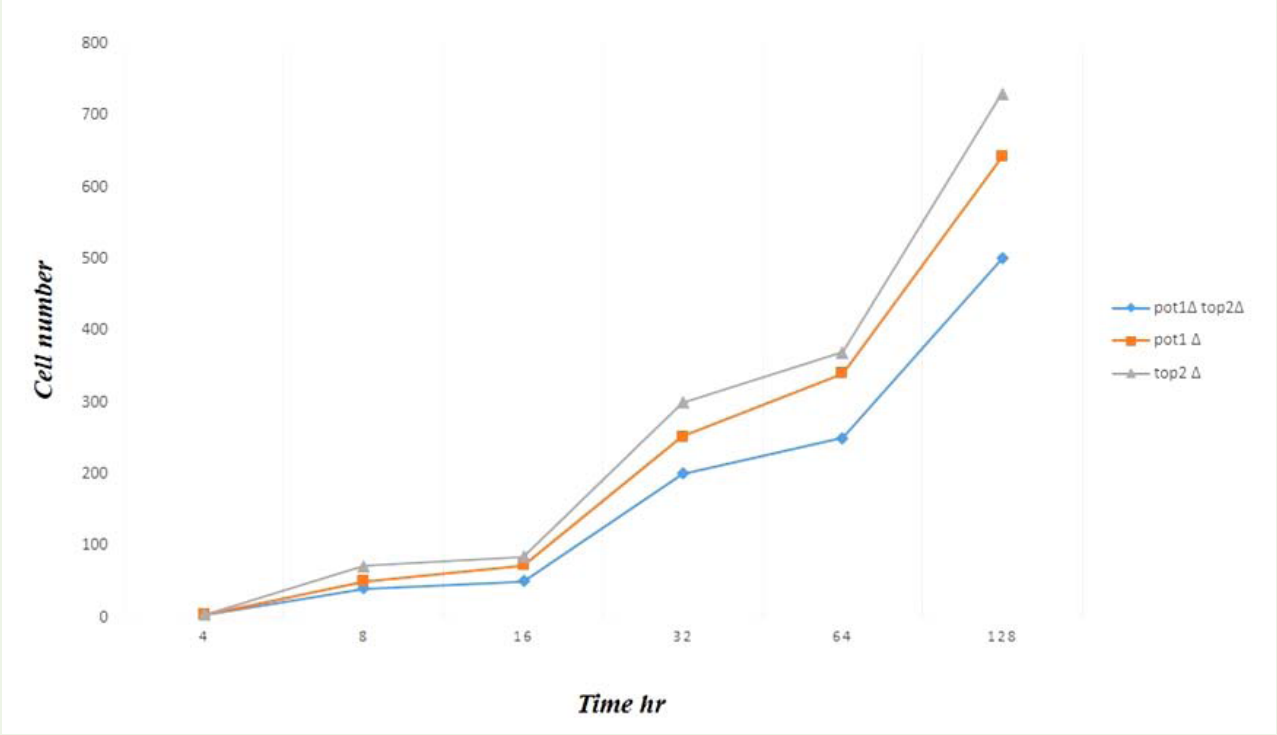

Figure 4: Cell counting result (C) showed that *pot1***Δ** *top2***Δ** have very low growth rate compare to *pot1***Δ** single mutant and *top2***Δ** single mutant as shown. The results are expressed as mean.

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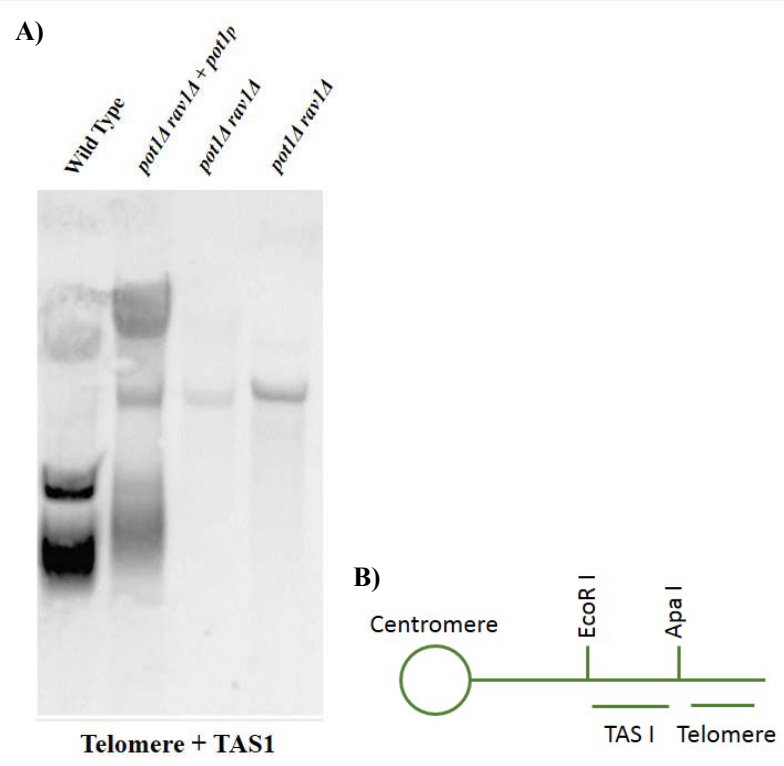

Figure 5: (A) *pot1***Δ** *rav1***Δ** double mutants are viable and lose telomeric DNA. The *pot1***Δ***rav1***Δ** double mutants were plated on EMM plus Adenine and Leucine (EMM+AL) and YEA+FOA at 25°C.The Pot1 plasmid is retained on EMM+AL and cells that lost the plasmid were selected against on YEA+FOA at 25°C. The telomere lengths of the *pot1***Δ** *rav1***Δ** double mutants were analyzed using Southern hybridization at 25°C *pot1***Δ** *rav1***Δ** cells that express Pot1 plasmid (+Pot1p) were used as controls that have telomeric DNA. Genomic DNA was digested with EcoRI and separated by 1.5% agarose gel electrophoresis. A telomere fragment (Telomere) plus telomere- associated sequence (TAS1) derived from PNSU70 [32] was used as a probe. To assess the total amount of DNA, the gel was stained with ethidium bromide (EtBr) beforeblotting onto the membrane was performed. (B) Restriction enzyme sites around the telomere and TAS1 of 1 chromosome arm cloned in the plasmid PNSU70 [32].

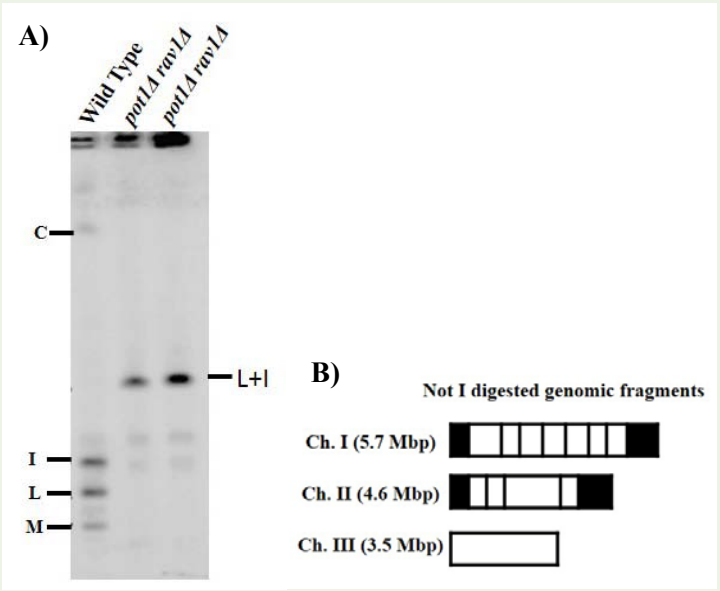

Figure 6: Chromosomes of the *pot1***Δ** *rav1***Δ** cells are circularized by single strand annealing (SSA).(A) NotI-digested *S. Pombe* chromosomal DNA from the wild type, a *pot1***Δ** isolate two independent *pot1***Δ** *rav1***Δ** isolates and one *pot1***Δ** *rav1*(+Pot1p) isolate were analyzed using PFGE. Probes specific for the telomericNotI fragments (M, L, I, C) were used [33]. (B) NotI restriction site map of *S. Pombe* chromosomes. Chromosomes I, II, and III (Ch. I, Ch. II, and Ch. III) are shown.

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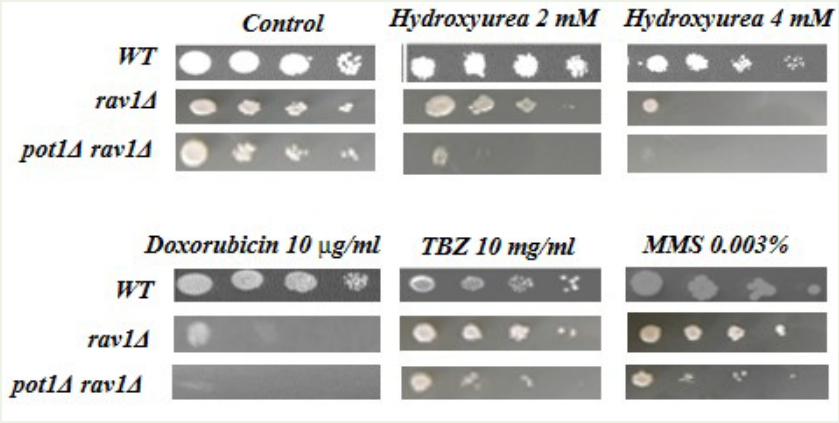

Figure 7: Spotting assay of ten-fold dilutions of cells. The *pot1***Δ** *rav1***Δ** double mutant (two candidates) that lost the plasmid and *rav1***Δ** as wild type working as a control were selected against on YEA+HU at final concentrations (2 mM and 4 mM), YEA +MMS at final concentrations (0.003% and 0.0015%), YEA+ Doxorubicin at final concentration (10 µg/ml), YEA+ TBZ at final concentration (10 mg/ml) and free YEA as control.

Results

The phenotypic of the *pot1*Δ *top2*Δ double mutant our result showed that after we streaked *pot1*Δ *top2*Δ double mutant, Wild type (W.T), *Pot1*Δ single mutant which lost for pot1 plasmid and *top2*Δ single mutant on YEA medium plate and incubated at different temperatures 25 °C, 30 °C. The result shows *pot1*Δ *top2*Δ is not viable at 30 °C, but *pot1*Δ and *top2*Δ is viable compare to W.T as a control Figure 1. Our results suggest that *top2* gene function required to maintain viability under these conditions. This might occur after the circularization and suggested that *top2* gene efficiency very low and disorders happened in chromosome segregation or DNA synthesis. If this were the case we must detect the problems during S phase by performed FACS analysis to detect the problems during DNA synthesis in addition to detect problems during M phase by performed DAPI stained.

FACS analysis

The analysis of cellular DNA content following cell staining with both propidium Iodide (PI) and deconvolution of the cellular DNA content frequency histograms. This approach reveals distribution of cells in three major phases of the cell cycle (G1, S, and G2/M). The data are presented as cellular DNA content frequency histograms. To obtain percentage of cells in S phase to detect the disorders in DNA synthesis during S- phase. Our results showed that the *pot1*Δ *top2*Δ double mutant cells have deficiency in DNA synthesis compare to *pot1* single mutant and *Top2* single mutant under these condition as shown in Figures 2,3,4.

Both candidates of *pot1*Δ *rav1*Δ double mutant lose telomeric DNA completely, and the chromosomes are circularized. The *pot1*Δ disruptant loses telomeric DNA completely, and survival depends on the circularization of chromosome via SSA [31]. We analyzed telomere length in the double mutants by using southern hybridization, both candidates of *pot1*Δ *rav1*Δ double mutant had lost telomeric DNA Figure 5. Next, we analyzed the chromosome structure by Pulsed Field Gel Electrophoresis (PFGE) Figure 6. The Not I-digested fragments M, L, I, and C, which are located in wild type cells. In contrast, the L+I band can be detected in both candidates of *pot1*Δ *rav1*Δ double mutant. This is similar to *pot1*Δ single mutant, which has circular chromosomes. This results demonstrate that the chromosomes of both the *pot1*Δ *rav1*Δ double mutants are circularized.

Sensitivity of *pot1*Δ *rav1*Δ double mutant to drugs The viability of a mutant of the gene encoding a regulator of assembly of V-ATPase (*rav1*Δ) and Both *pot1*Δ *rav1*Δ candidates showed hypersensitivity to Doxorubicin as reported before [34] at final concentration(10 µg/ml) and hydroxyurea (HU) at final concentration (4 mM) and slightly high sensitivity to hydroxyurea at final concentration (2 mM) and moderate sensitivity to thiabendazole (TBZ) at final concentration (10 mg/ml) and Miracle Mineral Solution (MMS) at final concentration (0.003% and 0.0015%) Figure 7.

Discussion

*pot1*Δ cells survival of the loss of telomere function is due to chromosome circularization. That is depended on SSA. It has been recently suggested that *S. Pombe top2* gene has a similar involvement. This led us to investigate the hyposis that *top2* gene required for the viability of *pot1Δ* cells Figure 1. FACS analysis results suggested that the unviability of *pot1*Δ *top2*Δ double mutant due to the disorders in DNA synthesis during S phase Figure 2. DAPI stained results gave us another hyposis for the unviability of the double mutant might due to the some problems happen during chromosome segregation during M phase Figure 3. In addition to the growth of the double mutant at 30°C gave us very clear evidence when the efficiency of *top2* gene was very low the *pot1*Δ cells couldn't grow.

top2 gene very important target for many new anticancer drugs this led us to developing a new assay system using *S. Pombe pot1*Δ *rav1*Δ double mutant to use these model for screening *top2* inhibitors. The mutation in *rav1* gene have increased the sensitivity of *pot1*Δ cells toward drugs as shown in Figure 7. This evidence gave us the important role of rav1 gene in drug efflux and influx.

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and Southern hybridization instruments at the Graduate School of Advanced Science of Matter and Graduate School of Biomedical and Health Science Hiroshima University.

References

1. Sipieki M (2000) Where does fission yeast sit on the tree of life?. Genome Biol 4: 1-1011.
2. Leupold U (1950) Die Verebung Von Homothallic and Heterothallic bie *Schizosaccharomyces Pombe*. C R Lab Carlsberg 24: 381-475.
3. Mitchison JM (1957) The growth of single cells: I. *Schizosaccharomyces Pobme*. Exp Cell Res 13: 244-262.
4. Fantes P, Beggs J (2000) The yeast Nucleus (oxford Univ Press, Oxford, 2000).
5. Daris L, Smith GR (2001) Meiotic recombination and chromosome segregation in *Schizosaccharomyces Pombe*. Proc Nat I Acad Sci USA 98: 8395-8402.
6. Humphrey T (2000) DNA damage and Cell cycle control in *Schizosaccharomyces Pombe*. Mutant. Res, 451: 211-226.
7. Lang BF, Cedergrn R, Gray MW (1987) The mitochondrial genome of the fission yeast, *Schizosaccharomyces Pobme*. Sequence of the large-subunit ribosomal RNA gene, comparison of potential secondary structure in fungal mitochondrial large- Subunit rRNAs and evolutionary considerations. Eur J Biochem 169: 527-537.
8. Devendra ST (2011) Topoisomerase II inhibitors in cancer treatment. International Journal of Pharmaceutical Sciences and Nanotechnology: 3.
9. Kellner U, Sehested M, Jensen PB, Gieseler F, Rudolph P (2002) Culprit and Victim- DNA topoisomerase II, Lancet Oncol 3: 235-243.
10. Hsieh TS, Brutlag D (1980) ATP-dependent DNA topoisomerase from D. melanogaster reversibly catenates duplex DNA rings. Cell 21: 115-125.
11. Baldi M, Beneditti P, Mattoccia, E, Tocchini-ValentiniGP(1980) In vitro catenation and decatenation of DNA and a novel eukaryotic ATP-dependent topoisomerase. Cell 20: 461-467.
12. Hartsuiker E, Bahler J, Kohli J (1998) The role of Topoisomerase II in meiotic chromosome Condensation and segregation in *Schizosaccharomyces Pombe*. Mol Biol Cell 9: 2739-2750.
13. Pommier Y, Leo E, Zhang H, Marchand C (2010) DNA topoisomerases and SWI/SNF and RSC complexes show compositional and functional differences their poisoning by anticancer and antibacterial drugs. Chem Biol 17: 421-433.
14. Ishida R, Hamatake M, Wasserman RA, Nitiss JL, Wang, JC, et al. (1995) DNA topoisomerase II is the molecular target of bisdioxopiperidine derivatives ICRF-159 and ICRF-193 in *Saccharomyces cerevisiae*. Cancer Res 55: 2299-2303.
15. Chen AY, Liu LF (1994) DNA topoisomerase essential enzymes and lethal targets. Annu Rev Pharmacol Toxicol 34: 191-218.
16. Roy C, Brown DL, Little JE, Valentine BK, Walker PR, et al. (1992) The topoisomerase II inhibitor teniposide (VM-26) induces apoptosis in unstimulated mature murine lymphocytes. Exp Cell Res 200: 416-424.
17. Endicott J, Ling V (1989) The biochemistry of P-glycoprotein-mediated multi drug resistance. Annu Rev Biochem 58: 137-171.
18. Issacs R, Harris AL, Hickson ID (1996) Regulation of the human topoisomerase II a gene promoter in confluence-arrested cells. J Biol Chern 271: 16741-16747.
19. Goswani PC, Roti Roti JL, Hunt CR (1996) The cell cycle-coupled expression of topoisomerase II during S phase is regulated by mRNA stability and is disrupted by heat shock or ionizing radiation. Mol Cell Biol 16: 1500-1508.
20. Nakajima T, Ohi N, Arai T, Nozaki N, Kikuchi A, et al. (1995) Adenovirue E1A-induced apoptosis elicits a steep decrease in the topoisomerase II a level during the latent phase. Oncogene 10: 651-662.
21. Nanbu T, Takahashi K, Murray JM, Hirata N, Ukimori S, et al. (2013) Fission Yeast RecQ Helicase Rqh1 Is Required for the Maintenance of Circular Chromosomes, Mol Cell Biol 336: 1175-1187.
22. Nakano A, Masuda K, Hiromoto T, Takahashi K, Matsumoto Y, et al. (2014) Rad51 dependent aberrant chromosome structures at telomeres and rDNA activate the Spindle assembly checkpoint. Mol Cell Biol 34: 1389-1397.
23. Pitt CW, Cooper JP (2010) Pot1 inactivation leads to rampant telomere resection and loss in one cell cycle. Nucleic Acids Res 38: 6968- 6975.
24. Wang X, Baumann P (2008) Chromosome Fusions following telomere loss are mediated by single-strand annealing. Mol Cell 31: 463-473.
25. Smith CL, Matsumoto T, Niwa O, Klco S, Fan JB, et al. (1987) An electrophoretic karyotype for *Schizosaccharomyces pombe* by pulsed field gel electrophoresis. Nucleic Acids Res 15: 4481-4491.
26. Parsons AB, Brost RL, Ding H, Li Z, Zhang C, et al. (2004) Integration of Chemical-genetic and genetic interaction data link, bioactive compounds to cellular target pathways. Nat Biotechnol 22: 62-69.
27. Yoshida S, Anraku Y (2000) Characterization of staurosporine-sensetive mutants of *Saccharomyces Cerevisiae:* Vacuolar functions affect staurosporine sensitivity. Mol Gen Genet 263: 877-888.
28. Dawson K, Toone WM, Jones N, Wilkinson CR (2008) Loss of Regulators of Vacuolar ATPase Function and Ceramide Synthesis Results in Multidrug sensitivity in *Schizosaccharomyces Pome*. Eukaryot Cell 926-937.
29. Baumann P, Cech TR (2000) Protection of telomeres by the Ku protein in fission yeast. Mol Biol Cell 11: 3265–3275.
30. Wang X, Baumann P (2008) Chromosome fusions following telomere loss are mediated by single-strand annealing. Mol Cell 31: 463-473.
31. Sugawara N (1988) DNA sequences at the telomeres of the fission yeast S. pombe. Ph.D. dissertation. Harvard University, Cambridge, MA.
32. Nakamura TM, Cooper JP, Cech TR (1998) Two modes of survival of fission yeast without telomerase. Science 282: 493-496.
33. Tay Z1, Eng RJ, Sajiki K, Lim KK, Tang MY, et al. (2013) Cellular Robustness Conferred by Genetic Crosstalk Underlies Resistance against Chemotherapeutic Drug Doxorubicin in Fission Yeast. 8: e55041.

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