Examining the involvement of Slx5 in the apoptotic response to chronic activation of the spindle assembly checkpoint

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1. Introduction

The spindle assembly checkpoint (SAC) is a conserved major cell cycle checkpoint, which ensures high fidelity of chromosome segregation in mitosis. SAC monitors kinetochore-microtubule attachments. In the presence of improper attachment(s), SAC activation leads to a cell cycle arrest in metaphase until the attachment error is corrected before the cell proceeds to anaphase, thus prevents aneuploidy in daughter cells (Lara-Gonzalez et al., 2012). Since SAC activation prevents cell proliferation by inducing a mitotic arrest in response to improper kinetochore-microtubule attachments, microtubules represent one of the most successful chemotherapeutic targets in clinic today. Two groups of anticancer drugs, taxanes (paclitaxel/taxol, docetaxel) and vinca alkaloids (vinblastine, vincristine) that are successfully used in the treatment of several types of cancer in clinic, prevent cancer proliferation by targeting microtubules (Hadfield et al., 2003; Marques et al., 2015). Both groups of drugs disrupt spindle microtubules, thus prevent proper attachments. Lack of proper attachments due to the drug treatment induces chronic SAC activation, which leads to a prolonged mitotic arrest (Gascoigne and Taylor, 2009). Since mitosis is considered the most vulnerable cell cycle phase to various environmental factors including radiation (Stobbe et al., 2002) and chemicals (Hughes, 1950), mitotic arrest induction (increasing the time cells spend in mitosis) is favorable for cancer treatment.

SAC-activating antimitotic drugs have been reported to induce a prolonged mitotic arrest in all cancer cell lines tested (Huang et al., 2010). Different cell fates have been identified following the prolonged mitotic arrest. Among these, “apoptosis” (programmed cell death) has been widely accepted as one of the main mechanisms through which anticancer drugs kill cancer cells (Atalay and Aydın, 2017). Recent studies revealed the link between chronic SAC activation and apoptosis by showing that McI1, a human antiapoptotic protein, is degraded by a Cdk1/cyclin B phosphorylation and APC/C dependent mechanism (Harley et al., 2010; Wertz et al., 2010). A DNA damage response mechanism activated the damage accumulated in mitotically arrested cells (Imre et al., 2011; Hayashi et al., 2012) has also been shown to induce apoptosis in these cells (Hain et al., 2016). However, the
nature of the signal produced as a result of chronic SAC activation and its relation to the apoptotic mechanism is largely unknown.

Apoptosis, a highly regulated mode of programmed cell death, is a vital component of normal development and aging, as it is essential for maintaining tissue homeostasis by balancing cell death and cell division. Although different apoptotic pathways require specific signals to be triggered, they all converge on the same execution pathway. Activation of the execution pathway leads to the apoptosis specific characteristics such as caspase activation, DNA fragmentation, and membrane blebbing (Elmore, 2007). Although apoptosis has been considered a metazoan programmed cell death mechanism, *Saccharomyces cerevisiae* has also been demonstrated to undergo cell death with apoptotic features over two decades ago (Madeo et al., 1997). Since then, several studies reported that yeast cells undergo apoptosis in response to various stress conditions including acetic acid, hydrogen peroxide, high salt or sugar (Madeo et al., 2004). Chronic SAC activation-induced prolonged mitotic arrest also triggers apoptosis in *S. cerevisiae* (Endo et al., 2010).

Slx5 (the yeast orthologue of human RNF4) is a subunit of the budding yeast heterodimeric Slx5/Slx8 sumo-targeted ubiquitin ligase complex. Sumoylation is a reversible posttranslational modification, which involves covalent attachment of small ubiquitin-related modifier (sumo) to the substrate lysine residues on target proteins (Flotho and Melchior, 2013). Several proteins involved in many key cellular processes including DNA damage repair, cell cycle, transcription, and chromosome segregation are known to be sumoylated. Sumoylation is important for response to various stress conditions such as heat shock, oxidative stress, DNA damage, and ethanol stress (Enserink, 2015), and has been shown to be associated with apoptosis in different cell lines (Sudharsan ve Azuma, 2012; Jin et al., 2017; Xiu et al., 2018), as well as *S.cerevisiae* (Owsianowski et al., 2008). Ubiquitination of the sumoylated proteins is critical for the turnover of the sumoylated proteins, because sumo-targeted ubiquitin ligases can specifically induce degradation of sumoylated proteins (Geoffroy and Hay, 2009). A large number of proteins are known to be sumoylated in *S.cerevisiae* as well: A proteomic analysis of sumoylation revealed a total of 251 sumo-conjugated proteins in yeast (Denison et al., 2005).

In this study, we investigated whether Slx5 is involved in the apoptotic response induced by chronic SAC activation. Our data revealed that chronic SAC activation induces a prolonged mitotic arrest in slx5Δ cells similar to wild type cells. However, the percentage of slx5Δ cells displaying apoptotic features of nuclear fragmentation, DNA fragmentation and intracellular ROS (reactive oxygen species) production following the chronic SAC activation was lower compared to wild type cells, suggesting that Slx5 may be involved in the chronic SAC activation-apoptosis relation. We also showed that the possible role of Slx5 in chronic SAC activation-apoptosis relation is not through ubiquitin dependent degradation of 3 apoptosis-related and sumoylated candidate proteins.

2. Materials and methods

2.1. Strains and growth conditions

All *Saccharomyces cerevisiae* strains (WT, slx5Δ and TAP-tagged strains) used in this study were in the BY4741 background (*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*). WT TAP-tagged strains were a kind gift from Dr. Daniel J. Burke (North Carolina State University, College of Sciences, Department of Biological Sciences). *slx5Δ* was deleted in each WT TAP-tagged strain by one-step gene replacement. *slx5α::KANMX* was amplified by PCR and transformed into the WT TAP-tagged strains by lithium-acetate transformation method as described previously (Gietz and Schiestl, 2007). All strains were maintained on YPD agar plates containing 1% (wt/vol) yeast extract, 2% (wt/vol) peptone, 2% (wt/vol) glucose, and 2% (wt/vol) agar and cultured in liquid YPD medium (2% wt/vol) glucose, 1% (wt/vol) yeast extract and 2% (wt/vol) peptone) shaking (175 rpm) at 30°C.

2.2. Chronic SAC activation

In order to induce chronic SAC activation, WT and slx5Δ strains were grown to early mid-logarithmic phase (OD600 = ~0.3) and arrested in G1 with α-factor (200 μM) in acidic medium (pH = 3.4) at 30°C for 2 h. G1 synchronization was considered successful when more than 90% of the cells were observed to be unbudded under the light microscope. G1–arrested cells were removed from α-factor containing medium by washing 3 times with dH2O and released into nocodazole containing medium (15 μg/ml) (0 h) and incubated for 10 h (10 h). Nocodazole (15 μg/ml) was readded 5 h after their release into the nocodazole-containing medium. Samples collected from both strains at every 2 h (0 h, 2 h, 4 h, 6 h, 8 h, 10 h) were examined for SAC activation, apoptotic features, and concentrations of TAP-tagged candidate proteins.

2.3. Western blot analysis

Protein extracts of samples taken from WT and slx5Δ cultures at every 2 h for 10 h were prepared by the NaOH method as described previously (Kushnirov, 2000). The whole cell extracts were separated on 10% SDS-containing polyacrylamide gel and transferred onto immobilon PVDF membrane (Millipore). The membranes, blocked in 5% dry fat-free milk in 1XPBS (phosphate-buffered saline) for at least 1 h at room temperature, were probed with anti-Pds1 (Santa Cruz), anti-Clb2 (Santa Cruz) for mitotic arrest detection, and with anti-TAP (Invitrogen) for the
detection of TAP-tagged proteins. Anti-Pgk1 (Abcam) was used for the detection of the loading control (Pgk1).

Following the primary antibody incubations overnight at 4°C, membranes were washed three times with 1XPBS and incubated with the appropriate HRP-conjugated secondary antibodies in 5% dry fat-free milk in 1XPBS for 1 h at room temperature. Following three washes with 1XPBS, the blots were stained with ECL (WesternBright Sirius HRP substrate, Advansta), exposed to MXBE blue film (Carestream) (for 5 s to 2 min, depending on the detected protein) and manually developed. Pictures of the films were taken on a light box. Pds1 and Clb2 band intensities were quantified using the ImageJ software (Scion Corp, Bethesda, Maryland, USA). The double band observed for Clb2 may be due to the detection of two splice variants of Clb2 by the anti-Clb2 antibody.

2.4. Flow cytometry analysis
Cells were fixed with 70% ethanol at room temperature and kept at 4°C until flow cytometry analysis. Prior to the analysis, the cells were rehydrated in water and treated with pepsin for 30 min at 37°C. Pepsin was removed from the samples by washing with dH₂O 3 three times. The cells were then treated with RNase at 37°C overnight. Cells were stained with sybr green for 5 min in the dark and were then treated with RNAse at 37°C overnight. Cells were stained with sybr green for 5 min in the dark and sonicated prior to the analysis. Flow cytometry analyses were performed on Accuri™ C6 flow cytometer (BD biosciences). A total of 40,000 cells at each time point were evaluated for DNA content.

2.5. DAPI (4′, 6-diamidino-2-phenylindole) staining
Samples collected at each time point were fixed with 3.7% (v/v) formaldehyde for 1.5 h at room temperature. Following the fixation, cells were washed twice with dH₂O, resuspended in dH₂O, and kept at 4°C until DAPI staining. For DAPI staining, samples were centrifuged and fixed with 70% (v/v) ethanol for 30 min at room temperature, washed once with dH₂O, and resuspended in dH₂O. Five μL of the samples were loaded onto microscope slides and stained with DAPI (4′, 6-diamidino-2-phenylindole) (2 μg/ml) in Vectashield mounting medium (Vector, Burlingame, CA). At least 200 cells for each time point were evaluated for nuclear fragmentation under a fluorescence microscope (Leica DM1000 LED, Leica Microsystems, Germany) and categorized as “fragmented” or “unfragmented”. The experiment was conducted three times for each strain and the average of the “percent fragmented nucleus” was reported with standard error of the mean.

2.6. TUNEL (Terminal deoxynucleotidyl transferase dUTP nick-end labeling) assay
DNA fragmentation was analyzed by TUNEL assay as described previously (Madeo et al., 1997) with some modifications. Briefly, samples were fixed with 3.7% (vol/vol) formaldehyde for 1-1.5 h at room temperature. Following the fixation, cells were washed with (PBS) three times, resuspended in dH₂O, and maintained at 4°C until the TUNEL staining. For TUNEL staining, yeast cell walls were digested with 24 μg/ml Zymolase 100T (MP Biomedicals) at 37°C for 1 h. Ten mL of the cell suspension was applied onto a polylysine-coated microscope slide and allowed to dry for 30 min at 37°C. After rinsed with PBS, the slides were permeabilized with 0.1% (vol/vol) Triton X-100 and 0.1% (wt/wt) sodium citrate for 2 min on ice, rinsed twice with PBS, and incubated with 10 μL TUNEL staining reaction mixture (in situ cell death detection kit, fluorescein, Roche diagnostics) at 37°C for 1 h in the dark and the slides were washed three times with PBS. The slides were mounted with a drop of Kaiser's glycerol gelatin (Merck) and approximately 200 cells at each time point were examined for TUNEL staining under a fluorescence microscope (Leica DM1000 LED, Leica Microsystems, Germany) and scored as “TUNEL positive” and “TUNEL negative”. The average “percent TUNEL positive cells” of three independent experiments was reported for each strain with standard error of the mean.

2.7. Detection of reactive oxygen species (ROS)
Intracellular ROS levels in each strain were examined as described previously (Madeo et al., 1999). Briefly, 200 μL samples taken at every time point were resuspended in fresh YPD and incubated with 10 μg/ml 2′, 7′-dichlorodihydrofluorescein diacetate (H2DCFDA; molecular probes) at 30°C for 40 min. Following the incubation, 5 μL of each sample was spotted onto microscope slides and observed under a fluorescence microscope (Leica DM1000 LED, Leica Microsystems, Germany) and scored as “positive” and “negative”. The experiment was conducted in triplicate for each strain and the average “percent DCF positive” cells were reported with standard error of the mean.

2.8. Statistical analyses
Each experiment that was analyzed statistically was repeated three times. Data represent standard error of the mean. Difference between the 0th and the 10th hour of the nocodazole incubation was examined using student’s t-test, the difference considered statistically significant when P < 0.05.

3. Results
3.1. Detection of the chronic SAC activation-dependent prolonged mitotic arrest
In order to investigate whether slx5Δ cells display a SAC-dependent prolonged mitotic arrest in response to long-term treatment with nocodazole, an antimicrotubule drug, slx5Δ and WT cells at early midlogarithmic phase
were synchronized in G1 with and released into nocodazole containing medium for 10 h to trigger chronic SAC activation. Samples collected from both strains at every 2 h (0 h, 2 h, 4 h, 6 h, 8 h, 10 h) were examined for Pds1 and Clb2 concentrations by western blot (Figure 1) and for DNA content by flow cytometry (Figure 2) analyses.

Degradation of Pds1, the anaphase inhibitor, is a widely accepted biochemical marker for metaphase-to-anaphase transition in *S. cerevisiae*. Pds1 levels reach their maximum in metaphase and decrease as the cells proceed into anaphase in cycling cells. SAC activation leads to stabilization of Pds1 levels due to metaphase-arrest induction (Yamamoto et al., 1996). Western blot analyses of Pds1 levels in WT and *slx5Δ* cells revealed that in *slx5Δ* cells, Pds1 was expressed 2 h after the α-factor release and remained stable during the course of the experiment similar to WT cells (Figure 1A, 1B), suggesting that *slx5Δ* cells arrest in metaphase in response to prolonged nocodazole treatment. Another biochemical marker for mitosis is the expression of Clb2, which is the yeast mitotic cyclin. Degradation of Clb2 is required for the cells to exit from mitosis (Wäsch and Cross, 2002). Consistent with the Pds1 results, Clb2 was expressed 2 h after the α-factor release and was not degraded even in the last time point (10th h) of the experiment in *slx5Δ* cells similar to WT cells (Figure 1A, 1C), indicating that *slx5Δ* cells stay in mitosis during the course of the experiment.

We also investigated mitotic arrest through examining the DNA content of single cells by flow cytometry. In support of the western blot results, flow cytometry analysis also showed that both *slx5Δ* and WT cells enter mitosis 2 h after the α-factor release with a 2C DNA content and maintain the 2C content during the course of the experiment (Figure 2). These data together indicate that *slx5Δ* cells arrest in mitosis in response to prolonged exposure to nocodazole, revealing that they have an intact SAC activity.

### 3.2. Examining apoptotic features during the prolonged mitotic arrest

WT budding yeast cells have been previously reported to undergo mitotic cell death with apoptotic features in response to prolonged (10-h) nocodazole treatment (Endo et al., 2010). After we observed that 10-h nocodazole treatment leads to a prolonged arrest in *slx5Δ* cells as well,
we investigated whether Slix5 is involved in the apoptotic response to the prolonged nocodazole treatment by comparing the proportion of WT and \( slix5\Delta \) cells displaying apoptotic features during the prolonged nocodazole treatment. For this purpose, α-factor-synchronized WT and \( slix5\Delta \) cells were incubated in nocodazole containing medium for 10 h. Samples taken from both cultures (0 h, 2 h, 4 h, 6 h, 8 h, 10 h) were examined for some apoptotic features.

Nuclear fragmentation is a morphology of apoptosis also in yeast (Madeo et al., 1997). Samples collected from WT and \( slix5\Delta \) cultures were investigated for their nuclear morphologies by DAPI staining and categorized as “intact” or “fragmented” under the fluorescence microscope (Figure 3A). We observed that percentage of cell with a fragmented nucleus (%Fragmented nucleus) in \( slix5\Delta \) was higher compared to that of WT at each time point: %Fragmented nucleus in \( slix5\Delta \) and WT strains at the 10th h of nocodazole incubation were 63.5% and 34.5%, respectively. However, is noteworthy that the ratio of cells with a fragmented nucleus in the \( slix5\Delta \) culture at the 0th h of the nocodazole treatment (30.5%) was also higher compared to WT at the same time point (4.1%) (Figure 3B). When we compared %Fragmented nucleus at the 10th h with the 0th h, we observed that %Fragmented nucleus in WT cells at the 10th hour (35.4%) was significantly higher compared to 0th hour (4.1%) (8.6-fold increase, \( P < 0.05 \)). However, in the \( slix5\Delta \) strain, there was no significant difference in the percentage of cells with fragmented nucleus between the 0th (30.5%) and 10th h (63.5%) of

![Figure 2. Flow cytometry analysis of SAC-dependent prolonged mitotic arrest. Synchronized WT and \( slix5\Delta \) cells were released into the nocodazole-containing media (0 h) and incubated for 10 h. DNA content of the samples were analyzed by flow cytometry. A total of 40,000 cells were evaluated at each time point.](image)

![Figure 3. Nuclear morphology by DAPI staining. (A) Nuclei of DAPI stained cells were categorized as “intact” or “fragmented” under the fluorescent microscope during the prolonged mitotic arrest. (B) At least 200 cells were examined at each time point and the ratio of cells with fragmented nuclei was graphed. Data represent the standard error of the mean of three independent experiments. *: \( P < 0.05 \).](image)
nocodazole treatment (2.1-fold increase, $P > 0.05$) (Figure 3B).

Fragmentation of DNA into oligonucleosomal size fragments is a typical morphology of yeast apoptosis as it is in mammals (Ribeiro et al., 2006). DNA fragmentation in WT and $slx5\Delta$ cells during the prolonged mitotic arrest was examined using the TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling) assay, which is based on labeling free 3’-OH ends of DNA fragments with fluorescent-conjugated dUTP, and the cells were categorized as “TUNEL positive” or “TUNEL negative” under the fluorescent microscope (Figure 4A). Similar to the DAPI results, %TUNEL Positive cells in WT cells at the 10th h (9.2%) was significantly higher compared to the 0th h (2.2%) (4.1-fold increase, $P < 0.05$), whereas in $slx5\Delta$ cells %TUNEL positive cells at the 10th h (6.3%) was not significantly different from the 0th h (1.9%) (3.2-fold increase, $P > 0.05$) (Figure 4B).

Reactive oxygen species (ROS) are important cell death regulators that are known to be associated with apoptotic pathways in S.cerevisiae as well as in complex eukaryotes (Carmona-Gutierrez et al., 2010). ROS accumulation in cells is known to be important in inducing apoptosis (Perrone et al., 2008). Therefore, we examined intracellular ROS production in response to prolonged nocodazole treatment in $slx5\Delta$ cells by the H$_2$DCFDA assay (Figure 5). The assay is based on the conversion of H$_2$DCFDA to a strong fluorescent DCF upon oxidation by ROS in the cells. Cells were categorized as “DCF negative” or “DCF positive” under the fluorescent microscope (Figure 5A) and %DCF positive cells were plotted (Figure 5B). Although %DCF positive cells in WT and $slx5\Delta$ strains were not statistically different at any time point during the experiment, %DCF positive cells were increased more in the WT strain at the 10th h of the nocodazole treatment compared to the 0th h (2.2-fold) relative to that of $slx5\Delta$ strain (1.7-fold) (Figure 5B).

3.3. Investigating the role of Slx5 in the chronic SAC activation-apoptosis relation

Our data indicate that Slx5 may be involved in the apoptotic response to prolonged mitotic arrest triggered by chronic SAC activation. Therefore, we finally tested whether the possible role of Slx5 in the chronic SAC activation-apoptosis relation is through its sumo-targeted E3 ubiquitin ligase activity. For this purpose, we examined and compared the concentrations of candidate proteins in WT and $slx5\Delta$ strains under chronic SAC activating (+NOC) conditions for 10 h by western blot analysis (Figure 6). Bmh1, Swi3, and Sli15 were selected as “candidates”, because these proteins are thought to be apoptosis-related by their own or their binding partner’s mutant phenotypes and were also shown to be sumoylated in yeast (Denison et al., 2005). We did not detect any dramatic differences in the concentrations of the candidate proteins between the two strains at any time point throughout the experiment (Figure 6). These data suggest that the role of Slx5 in the chronic SAC activation-apoptosis relation is not through ubiquitin dependent degradation of the candidate proteins.

4. Discussion

Antimitotics, one of the most effective group of drugs used in cancer therapy today (van Vuuren et al., 2015), trigger chronic SAC activation, which led to a prolonged mitotic arrest in all cancer cell lines tested (Huang et al., 2010). Cells can undergo different fates following the prolonged arrest. Among these fates, apoptosis is a major mechanism

![Figure 4](image-url). DNA fragmentation by TUNEL staining. (A) TUNEL stained cells were categorized as “TUNEL positive” or “TUNEL negative” under the fluorescent microscope. (B) For both WT and $slx5\Delta$ strains, at least 200 cells were evaluated for TUNEL staining and the ratio of TUNEL positive cells were graphed. Data represent the standard error of the mean of three independent experiments. *: $P < 0.05$. 
through which antimitotic drugs kill cancer cells (Mukhtar et al., 2014). Another outcome of the prolonged mitotic arrest, slippage, is an important factor that significantly reduces the effectiveness of chemotherapy (Matson and Stukenberg, 2011). Therefore, to be able to direct chronic SAC activation dependent prolonged mitotic arrest to the apoptosis outcome is important to increase chemotherapy effectiveness. Although induction of apoptosis following the prolonged mitotic arrest triggered by antimitotics is a desirable outcome of cancer therapy, the nature of the signal produced during the mitotic arrest and how it is related to the apoptotic mechanism is largely unknown.

Here we investigated the role of Slx5, a sumo-targeted E3 ubiquitin ligase, in the chronic SAC activation dependent prolonged mitotic arrest-apoptosis relation. Western blot analysis of Pds1 and Clb2 levels as well as flow cytometry analysis of the DNA content during the experiment revealed that chronic SAC activation triggered by a 10-h nocodazole incubation induces a prolonged mitotic arrest in both WT and slx5Δ cells (Figure 1), suggesting that Slx5...
is not required for chronic SAC activation. Our data on
WT cells support the previous study, which reported that
WT budding yeast cells arrest in mitosis in response to
chronic SAC activation induced by a 10-h nocodazole
treatment (Endo et al., 2010). Chronic SAC activation
dependent prolonged arrest in slx5Δ cells was reported
for the first time in the present study.

Over the last decades several studies revealed that S.
cerevisiae could undergo apoptosis with characteristic
markers of metazoan apoptosis (Carmona-Gutierrez
et al., 2010), which makes it a suitable model organism
to study apoptosis caused by antimotics. Endo et al.
previously reported that WT budding yeast cells die
in mitosis with apoptosis-like features in response to a
10-h treatment with nocodazole (Endo et al., 2010). In
this study, we investigated whether Slx5 is involved in
the apoptosis-like response to chronic SAC activation by
examining slx5Δ cells for apoptosis-related morphologies
such as nuclear fragmentation, DNA fragmentation,
and ROS production during chronic SAC activation
and comparing them to those of WT cells under the
same conditions. Consistent with Endo et al’s study, we
observed that prolonged mitotic arrest led to an increase
in the ratio of cells displaying apoptosis-related features
(fragmented nucleus, fragmented DNA, and ROS
accumulation) in the WT strain. Additionally, we show
the first time that the ratio of cells displaying the same
apoptotic features are lower in the slx5Δ strain under the
same conditions, suggesting that Slx5 may be necessary
for the chronic SAC activation to result in apoptosis.
Finally, we tested whether the role of Slx5 in the chronic
SAC activation-apoptosis relation is through its ubiquitin
ligase activity by candidate protein approach. We show
that the role of Slx5 is not through regulating ubiquitin
dependent degradation of the apoptosis-related and
sumoylated candidate proteins.

Although Slx5 is not required for the turnover of the
apoptosis-related candidate proteins during chronic SAC
activation, the involvement of Slx5 in the chronic SAC
activation-apoptosis relation through its ubiquitin ligase
activity cannot be completely ruled out. Slx5 could still
be mediating ubiquitin dependent degradation of other
apoptotic proteins that were not selected as a candidate
because they were either missed in the first literature
review or they hadn’t been shown to be sumoylated yet.

The role of Slx5 in the chronic SAC activation-
apoptosis relation may also be indirect. In other words,
Slx5 may be regulating ubiquitin dependent degradation
of proteins that are not indirectly involved in apoptosis.
Sumo targeted ubiquitin ligases are involved in several
important cellular processes including DNA damage
response (Dantuma and van Attikum, 2016). Slx5 is
also critical for preventing genomic instability as gross
chromosomal rearrangements, such as nonreciprocal
translocations and chromosome fusions, as well as
increased spontaneous DNA damage are increased in
slx5Δ cells (Zhang et al., 2006). However, recent studies
suggest that sumo-targeted ubiquitin ligases can prevent
or trigger genomic instability depending on the nature of
the DNA damage (Nie et al., 2017), and genomic instability
may induce apoptosis (Zhivotovsky and Kroemer, 2004).
Although it has been demonstrated that DNA damage
accumulates in cells during prolonged mitotic arrest
(Hain et al., 2016), not much is known about the nature of
the damage. In this respect, Slx5 may be involved in the
induction of apoptosis in response to the DNA damage
during the prolonged mitotic arrest, which may explain
why apoptosis is decreased in slx5Δ cells during chronic
SAC activation induced prolonged mitotic arrest. Further
studies are required to elucidate the role of Slx5 in the
relation between chronic SAC activation and apoptosis.

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References
Atalay PB, Aydin A (2017). Variation in cellular responses to anti-
mitotic drug-induced prolonged mitotic arrest. Abant Medical
Journal 6 (3): 134-143.

Carmona-Gutierrez D, Eisenberg T, Büttner S, Meisinger C,
Kroemer G et al. (2010). Apoptosis in yeast: triggers, pathways,
subroutines. Cell Death and Differentiation 17 (5): 763-73.

Dantuma NP, van Attikum H (2016). Spatiotemporal regulation of
posttranslational modifications in the DNA damage response.
EMBO Journal 35 (1): 6-23.

Denison C, Rudner AD, Gerber SA, Bakalarski CE, Moazed D et al.
(2005). A proteomic strategy for gaining insights into protein
sumoylation in yeast. Molecular and Cellular Proteomics 4 (3):
246-254.

Elmore S (2007). Apoptosis: a review of programmed cell death.
Toxicologic Pathology 35 (4): 495-516.

Endo K, Mizuguchi M, Harata A, Itoh G, Tanaka K (2010).
Nocodazole induces mitotic cell death with apoptotic-like
features in Saccharomyces cerevisiae. FEBS Letters 584 (11):
2387-2392.
Enserink JM (2015). Sumo and the cellular stress response. Cell Division 10: 4. eCollection 2015.

Flotho A, Melchior F (2013). Sumoylation: a regulatory protein modification in health and disease. Annual Review of Biochemistry 82: 357-385.

Gascoigne KE, Taylor SS (2009). How do anti-mitotic drugs kill cancer cells? Journal of Cell Science 122 (15): 2579-2585.

Geoffroy MC, Hay RT (2009). An additional role for SUMO in ubiquitin-mediated proteolysis. Nature Reviews Molecular Cell Biology 10: 564-568.

Gietz RD, Schiestl RH (2007). High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. Nature Protocols 2 (1): 31-34.

Hadfield JA, Ducki S, Hirst N, McGown AT (2003). Tubulin and microtubules as targets for anticancer drugs. Progress in Cell Cycle Research 5: 309-325.

Hain KO, Colin DJ, Rastogi S, Allan LA, Clarke PR (2016). Prolonged mitotic arrest induces a caspase-dependent DNA damage response at telomeres that determines cell survival. Scientific Reports 6: 26766.

Harley ME, Allan LA, Sanderson HS, Clarke PR (2010). Phosphorylation of Mcl-1 by CDK1-cyclin B1 initiates its Cdc20-dependent destruction during mitotic arrest. EMBO Journal 29 (14): 2407-2420.

Hayashi MT, Cesare AJ, Fitzpatrick JA, Lazzerini-Denchi E, Karlseder J (2012). A telomere-dependent DNA damage checkpoint induced by prolonged mitotic arrest. Nature Structural & Molecular Biology 19: 387-394.

Huang HC, Shi J, Orth JD, Mitchison TJ (2010). Cell death when the SAC is out of commission. Cell Cycle 9 (11): 2049-2050.

Hughes AF (1950). The effect of inhibitory substances on cell division; a study on living cells in tissue cultures. Quarterly Journal of Microscopical Science 91 (3): 251-277.

Imreh G, Norberg HV, Imre S, Zhivotovsky B (2011). Chromosomal breaks during mitotic catastrophe trigger γH2AX-ATM-p53-mediated apoptosis. Journal of Cell Science 124 (Pt 17): 2951-2963. doi: 10.1242/jcs.081612.

Jin L, Shen K, Chen T, Yu W, Zhang H (2017). SUMO-1 gene silencing inhibits proliferation and promotes apoptosis of human gastric cancer SGC-7901 cells. Cellular Physiology and Biochemistry 41 (3): 987-998.

Kushnirov VV (2000). Rapid and reliable protein extraction from yeast. Yeast (Chichester, England) 16 (9): 857-860.

Lara-Gonzalez P, Westhorpe FG, Taylor SS (2012). The spindle assembly checkpoint. Current Biology 22 (22): R966-980.

madeo F, Frohlich E, Frohlich KU (1997). A yeast mutant showing diagnostic markers of early and late apoptosis. Journal of Cell Biology 139 (3): 729-734.

Madeo F, Frohlich E, Ligr M, Grey M, Sigrist SJ et al. (1999). Oxygen stress: a regulator of apoptosis in yeast. Journal of Cell Biology 145 (4): 757-767.

Madeo F, Herker E, Wissing S, Jungwirth H, Eisenberg T et al. (2004). Apoptosis in yeast. Current Opinion in Microbiology 7 (6): 655-660.

Marques S, Fonseca J, Silva PM, Bousbaa H (2015). Targeting the spindle assembly checkpoint for breast cancer treatment. Current Cancer Drug Targets 15 (4): 272-281.

Matson DR, Stukenberg PT (2011). Spindle poisons and cell fate: a tale of two pathways. Molecular Interventions 11 (2): 141-150.

Mukhtar E, Adhami VM, Mukhtar H (2014). Targeting microtubules by natural agents for cancer therapy. Molecular Cancer Therapeutics 13 (2): 275-284.

Nie M, Moser BA, Nakamura TM, Boddy MN (2017). SUMO-targeted ubiquitin ligase activity can either suppress or promote genome instability, depending on the nature of the DNA lesion. PLoS Genetics 13 (5): e1006776.

Owsianowski E, Walter D, Fahrenkrog B (2008). Negative regulation of apoptosis in yeast. Biochimica et Biophysica Acta 1783 (7): 1303-1310.

Perrone GG, Tan SX, Dawes IW (2008). Reactive oxygen species and yeast apoptosis. Biochimica et Biophysica Acta 1783 (7): 1354-1368.

Ribeiro GF, Cörte-Real M, Johansson B (2006). Characterization of DNA damage in yeast apoptosis induced by hydrogen peroxide, acetic acid, and hypersomotic shock. Molecular Biology of the Cell 17 (10): 4584-4591.

Stobbe CC, Park SJ, Chapman JD (2002). The radiation hypersensitivity of cells at mitosis. International Journal of Radiation Biology 78 (12): 1149-1157.

Sudharsan R, Azuma Y (2012). The SUMO ligase PIAS1 regulates UV-induced apoptosis by recruiting Daxx to SUMOylated foci. Journal of Cell Science 125 (23): 5819-5829.

van Vuuren RJ, Visagie MH, Theron AE, Joubert AM (2015). Antimitotic drugs in the treatment of cancer. Cancer Chemotherapy and Pharmacology 76 (6): 1101-1112.

Wäsch R, Cross FR (2002). APC-dependent proteolysis of the mitotic cyclin Clb2 is essential for mitotic exit. Nature 418 (7061): 556-562.

Wertz IE, Kusam S, Lam C, Okamoto T, Sandoval W et al. (2010). Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature 471 (7336): 7336:110-114.

Xiu D, Wang Z, Cui L, Jiang J, Yang H et al. (2018). Suppression of genomic instability by SLX5 and SLX8 in Saccharomyces cerevisiae. DNA Repair (Amst) 56:752-762.