Supporting Information

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SI Materials and Methods

Yeast Strains. Fission yeast mutants were isogenic to the WT strain (smt0, *leu1*-32, and *ura4*-D18). The *rev1* gene was disrupted by *ura4*$^+$ transformation of a *ura4*-D18 strain using a construct in which PCR products upstream and downstream of the *rev1* ORF were fused to the *ura4*$^+$ gene using PCR (upstream homology: forward, TGCATCTGTAGATTCCTCCAC; reverse, CCCAAAACTCGTGAAGCC; downstream homology: forward, AAACCAGTCAATGATAAGCG; reverse, TAATCACATTTTATGATGGTCG). The presence of all null mutations used in this study was verified by PCR, with primers flanking the disruption. The *eso1*-D147N targeting construct was created by fusing two PCR products from the *eso1* gene with overlapping primers containing the point mutation (upstream homology: forward, TTGCTTTAGTATCTGGTAGTAGG; reverse, AATACTAGCCTTCTTAACC). The resulting mutant PCR product was then fused to the *KanMX6* gene and a downstream PCR product and transformed into WT cells (downstream homology: forward, CTAAGCGTTATATAGAAG; reverse, GTAATGAGAACTGCTCTTCC). Transformants were screened sequentially with PCR products flanking the targeting construct and then by sequencing of the point mutation region. The entire *eso1* gene was then sequenced to exclude transformants with PCR-related mutations. The resulting locus contains an insertion of the KanMX6 gene 39 bp downstream of the *eso1* stop codon. After crosses, the presence of the *eso1*-D147N point mutation was verified by sequencing.

Time-Lapse Microscopy. Microscopy was carried out as described previously (1). In brief, cells grown in YE6s medium (2) were spotted on a 35-mm 1% agar plate containing the same medium. The cell droplet was covered with a quartz slide and sealed with paraffin oil. Cells were maintained at 30 °C and imaged at 2-min intervals for 3 h, then irradiated and imaged until confluent. Images were calibrated with a 188-μM EM grid, and cleavage times and cell lengths were recorded manually.

Flow Cytometry. Fission yeast strains were grown to log phase in YE6s medium, then resuspended at 3 OD/mL. Then 5-μL spots of this resuspension were dried on the surface of YE6s/1% agar plates prewarmed to 30 °C. Dishes were incubated for 2 h, 30 min at 30 °C, then irradiated. The cells were further incubated at 30 °C until being harvested in 10 μL of YE6s and transferred into 1 mL of 70% ethanol. Flow cytometry was performed as described previously (3), except that SYTOX Green stain at 5 μM was used instead of DAPI. Haploid and diploid WT strains were included with each experiment as calibration standards, and their G2 populations were gated to give relatively pure 2C and 4C populations, respectively. DNA contents were measured by solving for DNA in the equation

$$\mathrm{FL1} = a + b[\mathrm{DNA}] + c[SSC], \qquad [1]$$

where FL1 is the green fluorescence reading and SSC is the side scatter. The parameters *a*, *b*, and *c* were determined from the calibration standards as follows. Linear regression analysis of the FL1-versus-SSC plot of the 2C standard provided the parameter, *c*, which is simply the slope of this regression. This factor takes into account the component of FL1 that is dependent on cell size and independent of DNA content. The *a* and *b* parameters were manually adjusted until the 2C and 4C standards had average DNA contents of 2.0 and 4.0, respectively, when calculated from Eq. (**1**); for instance, the experiment reported in Fig. 3*C* had parameter values of

$$a = 35[\mathrm{FL1}],$$
$$b = 170[\mathrm{FL1/C}],$$

and

$$c = 0.30[\mathrm{FL1/SSC}].$$

Using these parameters, it can be deduced that in a 2C population with an average SSC value of 400, 69% of green fluorescence results from DNA staining, 24% results from length-dependent fluorescence, and 7% results from background. Applying this method to measure the mean DNA content of 10 biological replicates of strain AJC-E41 produced an SD of 0.076 C. The signal-to-noise (S/N) ratio for multiple samples can be calculated using the following statistical definition:

$$\mathrm{S/N} = \mu/\sigma = 2.0\mathrm{C}/0.076\mathrm{C} = 26,$$

where μ is the the expected value and σ is the SD.

The S/N ratio can also be calculated for a single sample if one assumes that each cell in a gated 2C population has a DNA content of 2.0. The SD of the calculated DNA contents of such a population from the $t = 0$ time point in Fig. 3*C* is 0.12 C, giving an S/N of 2.0 C/0.12 C = 17. For a diploid 4C population, the SD of calculated DNA contents is 0.10 C, giving an S/N ratio of 4.0 C/0.10 = 40. Therefore, as cells progress through S phase from 2C to 4C, a doubling of the S/N ratio most likely occurs.

Irradiation. UV irradiation (254 nm) was delivered from a Stratalinker (Stratagene) and attenuated with a neutral density filter (5% transmittance). In flow cytometry experiments, doses of ≥50 J/m^2 were not attenuated, so as to speed up the protocol.

CPD Measurement. ^{14}C-labeled TT, TC, and TU CPD standards were prepared as described previously (4). A fission yeast strain engineered to uptake thymidine (5) was inoculated into 10 mL of EMM6s (2) containing 100 μCi of ^{14}C-labeled thymidine (CFA219; Amersham). The culture was grown to late log phase, then resuspended at 1.6 OD/mL in YE6s. Then 60-μL aliquots of this resuspension were spread on 35-mm Petri dishes containing 5 mL of YE6s/1% agar and then dried at room temperature to give a monolayer. For each UV dose, nine plates were UV irradiated (with the exception of the 100 J/m^2 dose, for which three plates were used, to conserve material), immediately after which cells were harvested in 1 mL of 70% ethanol and stored on ice. The cells were then resuspended in 1 mL of 70% ethanol to remove residual YE6s and stored overnight at 4 °C. The cells were centrifuged, and three samples were combined by resuspension in 1 mL of 50 mM sodium citrate (the 100 J/m^2 samples were not combined at this point), resuspended in 200 μL of 50 mM sodium citrate containing 15 μg of RNase A, and incubated for 2 h at 37 °C. After digestion, 1 mL of TE1X plus 1% SDS was added to each sample, after which the samples were incubated for 15 min at 55 °C. In a separate experiment, it was determined that the radioactivity remaining in the cells at this point copurifies with DNA and thus does not contain free thymidine. The cells were resuspended in 1 mL of 98% formate and 100 μL of water and the hydrolyzed for 4 h at 145 °C. In a separate experiment, it was verified that the hydrolysis of thymidine to thymine goes to completion under these conditions whereas CPD standards remain unaffected. The hydrolysate was centrifuged at 30,000 × *g* for 20 min, after which the supernatant was evaporated, resuspended in 5 μL 50% formate, spotted onto PEI cellulose sheets (EMD Chemicals), and developed in 85% 2-propanol. The TLC plates were imaged with a phosphorimager,

and the thymine region and the region containing TT, TC, CT, and TU dimers were cut out for scintillation counting.

Calculation of the Number of Lesions per Replicon. The distribution of interorigin distances in fission yeast is that expected from a Poisson-distributed random variable (6). Estimates of the average replicon size range from a low of 29 kb to a high of 120 kb (6, 7). A UV dose of 25 J/m^2 introduces a dimeric photoproduct about every 1.5–2.5 kb on average; therefore, a replicon must be at least 15–25 kb in size to contain an average of 10 lesions. The fraction of the genome covered by replicons of this size can be calculated from the exponential probability density function for interorigin distances as follows:

$$\Pr(S) = ae^{-aS}dS,$$

where Pr(S) is the probability that a replicon is a given size, S, and a is the average origin freqeency (origins/Kb).

The average amount DNA in replicons of a given size can be calculated as

$$\mathrm{DNA} = S \times \Pr(S) = S \times ae^{-aS}dS. \quad \textbf{[2]}$$

To determine which fraction of the genome is covered by replicons of size of S_1 or greater, Eq. (**2**) must be integrated and normalized as follows:

$$\begin{aligned} GenomeFraction(\geq S_1) &= \frac{\int_{S_1}^{\infty} Sae^{-aS}dS}{\int_0^{\infty} Sae^{-aS}dS} = \frac{\frac{-e^{-aS}}{a}(aS+1)\big|_{S_1}^{\infty}}{\frac{-e^{-aS}}{a}(aS+1)\big|_0^{\infty}} \\ &= \frac{\frac{-e^{-a\times\infty}}{a}(a\times\infty+1)+\frac{e^{-aS_1}}{a}(aS_1+1)}{\frac{-e^{-a\times\infty}}{a}(a\times\infty+1)+\frac{e^{-a\times0}}{a}(a\times0+1)} \\ &= \frac{\frac{e^{-aS_1}}{a}(aS_1+1)}{\frac{e^{-a\times0}}{a}(a\times0+1)} = \frac{\frac{e^{-aS_1}}{a}(aS_1+1)}{\frac{1}{a}} \\ &= e^{-aS_1}(aS_1+1) \end{aligned} \quad \textbf{[3]}$$

Using Eq. (**3**), we can calculate that a UV dose of 25 J/m^2 would introduce at least 10 lesions per replicon over 79%–90% of the genome, assuming an average replicon size of 29 kb and lesion densities from one lesion every 1.5~2.5 kb. Assuming an average replicon size of 120 kb and the same lesion density range, a UV dose of 25 J/m^2 would introduce at least 10 lesions per replicon in 98%–99% of the genome.

1. Callegari AJ, Kelly TJ (2006) UV irradiation induces a postreplication DNA damage checkpoint. *Proc Natl Acad Sci USA* 103:15877–15882.
2. Moreno S, Klar A, Nurse P (1991) Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol* 194:795–823.
3. Muzi Falconi M, Brown GW, Kelly TJ (1996) CDC18$^+$ regulates initiation of DNA replication in *Schizosaccharomyces pombe*. *Proc Natl Acad Sci USA* 93:1566–1570.
4. Birnboim HC, Nassim A (1974) Detection of pyrimidine dimers in hydrolysates of yeast DNA by high-voltage paper electrophoresis. *Mol Gen Genet* 130:291–296.
5. Hodson JA, Bailis JM, Forsburg SL (2003) Efficient labeling of fission yeast *Schizosaccharomyces pombe* with thymidine and BUdR. *Nucleic Acids Res* 31:e134.
6. Patel PK, Arcangioli B, Baker SP, Bensimon A, Rhind N (2006) DNA replication origins fire stochastically in fission yeast. *Mol Biol Cell* 17:308–316.
7. Heichinger C, Penkett CJ, Bähler J, Nurse P (2006) Genome-wide characterization of fission yeast DNA replication origins. *EMBO J* 25:5171–5179.

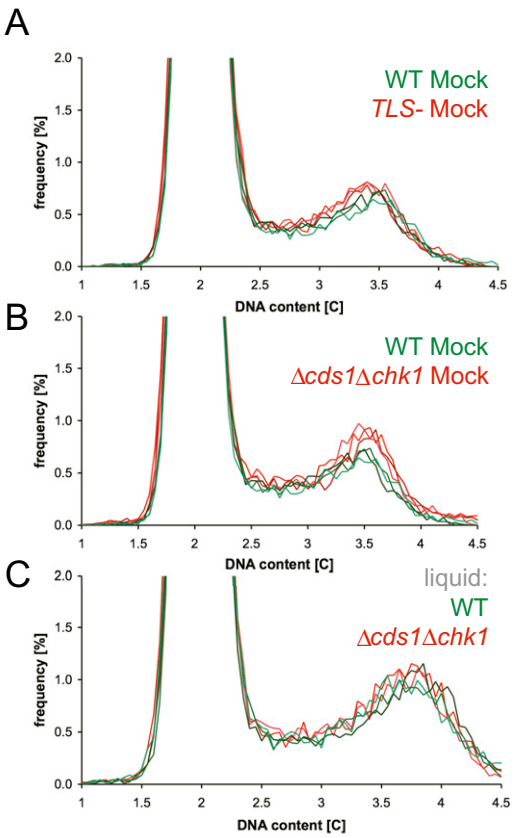

Fig. S1. A comparison of the DNA content distributions of nonirradiated cells with the indicated genotypes. Three independent experiments are superimposed in three shades of red or green. (*A* and *B*) Comparison of the mock-irradiated samples, grown on the surface of agar plates, from the experiment summarized in Fig. 4*C*. As noted in the text, the Δ*cds1*Δ*chk1* mutant has significantly more cells with a high DNA content than WT cells. (*C*) DNA content distributions from cells grown in liquid culture. In this case, there is no significant difference between WT and Δ*cds1*Δ*chk1* cells, because the WT strain has more high-DNA content cells than those shown in *A*. These observations can be explained if *cds1* and *chk1* reduce the rate of S phase in response to subtle environmental cues when cells are on the surface of an agar plate; however, this explanation requires further confirmation.

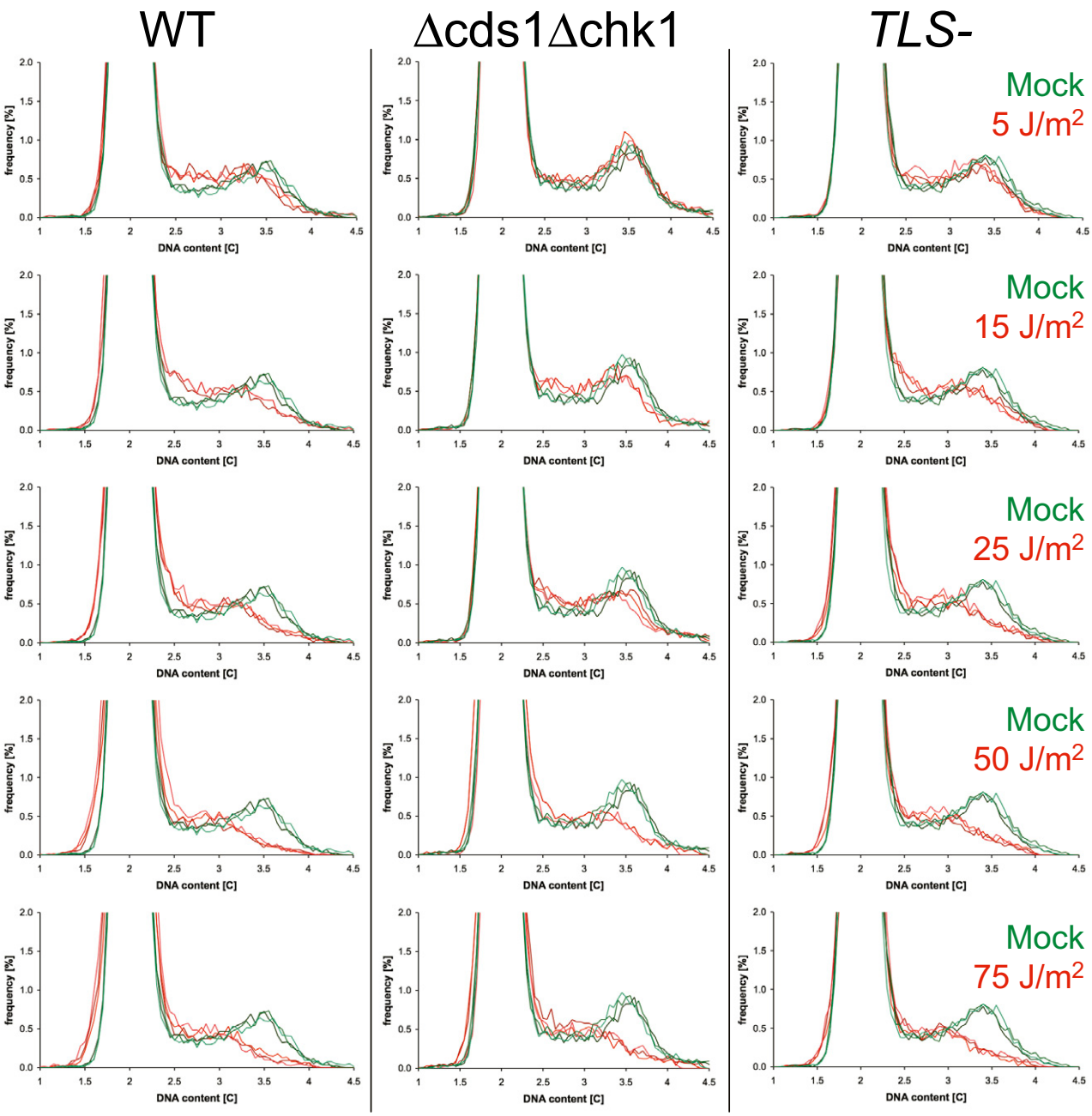

Fig. S2. DNA content distributions for the flow cytometry data summarized in Fig. 4*C*. Cells were mock-irradiated or irradiated with the indicated UV dose and harvested for flow cytometry 10 min later. In each case, the UV-irradiated distributions are superimposed on the mock-irradiated distributions. Three separate experiments are plotted in shades of green or red. UV doses of ≥100 J/m^2 produced distributions similar to 75 J/m^2 and thus were omitted to save space.

Table S1. *S. pombe* strains used in this study

Strain	Genotype	Source
AJC-E41	*smt0 ura4-D18 leu1-32*	Lab stock
AJC-G9	*smt0 leu1-32*	Lab stock
AJC-E1	*smt0 eso1::eso1-D147N-kanMX ura4-D18 leu1-32*	Present study
AJC-E56	*smt0 kpa1::kanMX ura4-D18 leu1-32*	Holmberg et al. (1)
AJC-E58	*smt0 rev3::kanMX ura4-D18 leu1-32*	Kai and Wang (2)
AJC-F53	*smt0 rev1::ura4+ ura4-D18 leu1-32*	Present study
AJC-G28	*smt0 eso1::eso1-D147N-kanMX kpa1::kanMX rev3::kanMX rev1::ura4+ ura4-D18 leu1-32*	Present study
AJC-G3	*smt0 cds1::ura4+ chk1::ura4+ ura4-D18 leu1-32*	Present study
FY2317	*h+ leu1-32::hENT1-leu1+ his7-366::hsv-tk-his7+ ura4-D18 ade6-M210*	Hodson et al. (3)

1. Holmberg C, et al. (2005) Ddb1 controls genome stability and meiosis in fission yeast. *Genes Dev* 19:853–862.
2. Kai M, Wang TS (2003) Checkpoint activation regulates mutagenic translesion synthesis. *Genes Dev* 17:64–76.
3. Hodson JA, Bailis JM, Forsburg SL (2003) Efficient labeling of fission yeast Schizosaccharomyces pombe with thymidine and BUdR. *Nucleic Acids Res* 31:e134.