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Shelterin components mediate genome reorganization in response to replication stress

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The natural dynamic of genome organization impacts critical nuclear functions including the regulation of gene expression, replication, and DNA damage repair. Despite significant progress, the mechanisms responsible for reorganization of the genome in response to cellular stress, such as aberrant DNA replication, are poorly understood. Here, we show that fission yeast cells carrying a mutation in the DNA-binding protein Sap1 show defects in DNA replication progression and genome stability and display extensive changes in genome organization. Chromosomal regions such as subtelomeres that show defects in replication progression associate with the nuclear envelope in sap1 mutant cells. Moreover, high-resolution, genome-wide chromosome conformation capture (Hi-C) analysis revealed prominent contacts between telomeres and chromosomal arm regions containing replication origins proximal to binding sites for Taz1, a component of the Shelterin telomere protection complex. Strikingly, we find that Shelterin components are required for interactions between Taz1-associated chromosomal arm regions and telomeres. These analyses reveal an unexpected role for Shelterin components in genome reorganization in cells experiencing replication stress, with important implications for understanding the mechanisms governing replication and genome stability.

Results

Defect in the Sap1 Protein Affects Genome Organization. Sap1 is an abundant nuclear protein that binds specific DNA elements distributed across the genome (17, 18). Multiple functions for Sap1 have been proposed, including a role in replication fork blocking (19–21), replication checkpoint activation (22), and genome organization (17). To gain further insight, we examined Sap1 subcellular localization. As previously shown (17), the Sap1 signal is concentrated within the nucleus in a diffuse pattern (Fig. S1A);
however high-resolution microscopy revealed that Sap1 forms a matrix-like pattern (Fig. L4). This localization was particularly interesting given the widespread distribution of Sap1 across the genome, including at solo LTRs and 13 copies of full-length Tj2 retrotransposons (18), which are organized into nuclear foci (called “Tj bodies”) in close proximity to the nuclear envelope (NE) (23, 24).

We considered that Sap1 binding to Tj2 might be required for Tj body organization. To explore this possibility, we used a partial loss-of-function mutant, sap1-1 (22). At restrictive temperature, sap1-1 showed loss of Sap1 nuclear signal (Fig. S1A). Unlike the widely distributed WT Sap1 showing distinct peaks at nucleosome-free regions, the mutant protein was stable but unable to bind broadly across the genome (Fig. S1 B–D). ChIP analysis showed depletion of the mutant Sap1 from Tj2 (Fig. 1B and Fig. S1C) correlating with an increase in Tj2 foci in sap1-1 cells (Fig. 1C), suggesting that Sap1 may have a role in clustering Tj2 into Tj bodies.

Defects in Tj body organization in sap1-1 cells might reflect broader changes in genome organization. We tested this possibility by performing FISH analysis of the rnt1 locus, which was enriched for Sap1 binding in WT cells but was depleted in sap1-1 cells (Fig. 1B). Compared with WT cells, the radial positioning of rnt1 relative to the NE protein Bqt4 (25) was shifted more to the nuclear interior in sap1-1 cells (Fig. 1D). Taken together, our results suggest that a defect in Sap1 affects genome organization, as indicated by disruption of Tj bodies and the altered positioning of other chromosome loci.

Nuclear Peripheral Association of Regions Containing Replication Origins in the sap1 Mutant. Our results prompted us to perform a global analysis of genome positioning with respect to the nuclear periphery. To examine peripheral contacts, we performed ChIP-chip analysis of the nuclear membrane marker GFP-Bqt3 (25) and compared the contacts made with chromosome regions in WT and sap1-1 cells. Importantly, GFP-Bqt3 decorated the NE in both WT and sap1-1 cells (Fig. 1E). In agreement with the Rabl rearrangement, specific regions of chromosomes associated with GFP-Bqt3. For example, GFP-Bqt3 was preferentially enriched at the centromere cores and telomeric regions of all three chromosomes (Fig. S2A and B). In addition, we observed Bqt3 enrichment at rRNA clusters located at the heterochromatin boundaries of centromere 2 (Fig. S2B). Interestingly, GFP-Bqt3 remained associated with centromeres and telomeres in sap1-1 cells and gained association with extended subtelomeric domains (>100 kb from each telomere) of chromosomes 1 and 2 but not chromosome 3 (Fig. 1F and Fig. S2C). Moreover, several regions of the chromosome arm showed GFP-Bqt3 enrichment in sap1-1 cells, indicating a newly formed association with the nuclear periphery (Fig. 1F and Fig. S2C), whereas other regions, such as rRNA clusters, lost association with GFP-Bqt3 (Fig. S2B). These results demonstrate genome-wide changes in the contacts made with the NE in sap1-1.

Strikingly, we noticed that most regions that gained association with GFP-Bqt3 contained DNA replication origins (Table S1). The newly formed genomic contacts at extended subtelomeric domains coincided with late origin cluster zones (Fig. 1F and Fig. S2C) (11). The specific association of selected chromosome regions containing origins with the nuclear periphery in sap1-1 cells suggested a possible connection between DNA replication activity and genome reorganization.

The sap1 Mutant Shows DNA Replication Defects. We next examined if sap1-1 affects DNA replication. To do so, we used the cdc10-v50 mutant to synchronize cells and monitor DNA replication progression from G1 arrest. FACS analysis revealed that sap1-1 cells spent a comparatively longer time in S-phase than WT cells (Fig. 2A), suggesting possible defects in replication in the mutant cells. To test for such defects, we examined the genome-wide replication profile by measuring BrdU incorporation in WT and sap1-1 cells released from G1 arrest in the presence of hydroxyurea (HU). As expected, efficient firing of replication origins occurred in WT cells (Fig. S3A). However, BrdU incorporation in sap1-1 cells was inefficient, particularly at subtelomeric regions containing clusters of late-replication origins that showed replication in this experimental set-up involving cdc10-v50 (Fig. 2B and Fig. S3B). Some chromosomal arm regions that showed association with GFP-Bqt3 in sap1-1 cells also showed low BrdU enrichment (Fig. 2B).

To investigate the effect of sap1-1 on replication further, we examined its impact on the genome-wide distribution of Mcm6 protein during S-phase. Mcm6 is a component of the MCM (mini chromosome maintenance) complex, which is a putative DNA replicative helicase required for replication initiation and elongation (26). We found a striking reduction of Mcm6 in sap1-1 cells, particularly between replication origins, as is consistent with abnormal replication progression (Fig. 2C and Fig. S3 C and D). Thus, global replication defects are indeed associated with loss of Sap1 function; however, the exact mechanism remains unknown.

sap1 Mutant Cells Accumulate ssDNA and DNA Damage-Repair Foci. Cells that experience replication stress tend to accumulate ssDNA, which can lead to genome instability (27, 28). To detect ssDNA, we quantified the number of Rad11 foci. Rad11 is a component of an ssDNA-binding complex called “replication protein A” (RPA), which is involved in DNA replication and/or DNA damage repair (28). Generally, WT S-phase cells have multiple faint Rad11 signals, and some mononucleated cells form a discrete single Rad11 focus in the nucleolus. However, we observed a significantly higher
number of sap1-1 cells than WT cells displaying two or more Rad11 foci in the chromatin hemisphere (Fig. 2D and Fig. S44). Moreover, time-lapse microscopy revealed that sap1-1 cells with Rad11 foci enter mitosis, resulting in the fragmentation of chromosomes (Fig. 2E, Fig. S48, and Movies S1 and S2). This result indicates that replication stress may be a potential source of genome instability in sap1-1 cells.

The sap1-1 Genome Contains Rearrangements. Replication defects and DNA damage in sap1-1 cells could cause genome instability, such as chromosomal rearrangements. Interestingly, sap1-1 cells frequently produced revertants capable of growing at an otherwise nonpermissive temperature (37 °C) (Fig. 3A). Microarray comparative genome hybridization (CGH) analysis of a revertant showed amplification of the region encompassing the sap1-1 locus (Fig. 3B and Fig. S54). We confirmed the duplication of this region, which resulted in a slight increase in Sap1 mutant protein (Fig. S55). The boundaries of the amplified region contain wfs (repeats often associated with Tj LTRs) bound by Sap1 in WT cells (Fig. 3B). Junction PCR analysis and subsequent Sanger sequencing revealed that the copy number gain resulted from direct tandem-oriented duplication (Fig. 3C and Fig. S5 C and D).

This rearrangement likely confers a survival advantage from the amplification of the sap1-1 region and suppression of the mutant phenotype. However, we found a more widespread destabilizing effect that involved other repeat structures, including wfs in other parts of the sap1-1 genome. In addition to wfs that flank sap1, recombination occurred between other tandem copies of wfs (such as wfs18–wfs13) in sap1-1 cells cultured at a semipermissive temperature (33 °C) (Fig. 3C). We also detected rearrangements in the subtelomeric repeats (Fig. S5E). These results clearly show that sap1-1 is prone to more widespread genome instability.

Because substrates for recombination can be generated by stalled or collapsed replication forks (29), we looked for replication defects at wfs elements. Indeed, 2D gel analysis revealed prominent replication fork pausing at the wfs9 region in sap1-1 cells (Fig. 3D). We also found that Rad52 was required for tandem duplication mediated by wfs repeats (Fig. S5F). Strikingly, sap1-1 cells lacking the well-defined checkpoint effector kinases Chk1 or Cds1 showed increased rearrangements (Fig. 3 A and E), suggesting that components of DNA damage and replication checkpoints are critical for suppressing genome instability in sap1-1 cells.

We also tested whether de-repression of wfs elements could be involved in promoting rearrangement. The histone deacetylases Chr3 and Chr6 have been implicated in the repression of wfs (30). However, defects in the histone deacetylases had no effect on wfs-mediated genomic rearrangements (Fig. S5G). Therefore, de-repression of wfs alone is not sufficient to trigger rearrangements. Rather, genome instability in sap1-1 is linked to defective replication
and DNA damage repair and is also accompanied by alterations in 3D genome organization.

**Genome-Wide Chromosome Conformation Capture Analysis Reveals Specific Interactions in the sap1-1 Mutant.** To obtain a detailed view of genome contacts in sap1-1, we performed genome-wide chromosome conformation capture (Hi-C) analyses. Two biological replicates were generated for both WT and sap1-1 cells. The Hi-C contact maps were highly reproducible. We found that previously described features of genome organization such as centromere and telomere clusters and heterochromatin-mediated intra- and interchromosomal arm interactions observed within centromere proximal regions were unaffected in sap1-1 cells (Fig. 4A and Fig. S6C). Scaling analysis revealed a slow decay in contact probability at distances <100 kb followed by a faster decay in sap1-1 cells, as in WT cells (Fig. S6B), indicating the existence of globules. Indeed, cohesin-dependent globules along chromosome arms were evident in sap1-1 cells, and the depletion of contact frequency (insulation) between regions separated by cohesin-bound globule boundaries was not affected (Fig. S6C). Consistently, the binding profile of cohesin subunit Psc3 was unchanged in sap1-1 cells (Fig. S6D).

Strikingly, our analyses revealed several prominent new contacts in the sap1-1 mutant that were not visible in WT cells. These contacts could be detected in sap1-1 cells cultured at semipermissive temperature for only 6 h. A common feature among all new contacts was the involvement of telomeric regions of chromosomes 1 and 2 (Fig. 4B). We observed newly formed contacts between the subtelomeric regions of the two short arms (tel1R and tel2L) and all three centromeres (Fig. 4B). Intriguingly, we also observed prominent interactions between telomeres and specific arm regions (Fig. 4B). These interactions were mainly restricted to the arms of chromosomes 1 and 2 and were not observed on chromosome 3 (Fig. S6E).

We further validated the new contacts using live-cell microscopy and chromosome conformation capture (3C) assays. Time-lapse

![Fig. 4](image-url)
microscopy revealed an association between centromeres and telomeres in a significant proportion of sap1-1 cells (Fig. 4C and Movies S3 and S4), in contrast to WT cells (Movies S5 and S6). Moreover, our 3C experiment detected an interaction between a region on the chromosome 1 arm (the genomic position of the 4,500- to 4,650-kb region) and tel1R in sap1-1 cells, which are ~1 Mb apart in linear genomic distance (Fig. 4D). This interaction was specific to sap1-1 cells and was detected only when cells were cultured at semipermissive temperature.

To exclude the possibility that our 3C experiments detected genomic rearrangements rather than new interactions, we performed PCR analysis using genomic DNA from sap1-1 cells. Importantly, no PCR amplification could be observed (Fig. S6F). Based on these results, we conclude that sap1-1 mutant cells, which show replication defects and genome instability, undergo genome reorganization resulting in specific new interactions with telomeres.

Shelterin Mediates New Interactions. We noted that many of the arm regions that contacted telomeres contained previously described late origins that are bound by Taz1 (Fig. S6E) (11, 12). Notably, Taz1 peaks were generally observed at the edges rather than at the center of these interacting regions. This observation may be a consequence of our Hi-C analyses that excluded Taz1-bound repetitive telomeric sequences, which might interact directly with chromosomal internal sites showing Taz1 peaks. Thus, the detected interactions most likely reflect contacts between distal sequences neighboring direct interaction sites (i.e., telomeres and Taz1-associated arm regions).

We investigated whether Taz1–Shelterin affects interactions between telomeres and arm regions. To do so, we performed 3C analyses in sap1-1 cells lacking Taz1 or other Shelterin subunits such as Rap1 or Ccq1. Remarkably, the loss of any of these factors significantly reduced the interaction between tel1R and a Taz1-associated arm region (chromosome 1: 4,500- to 4,650-kb region) that contains late origins and associates with the nuclear periphery in sap1-1 cells (Fig. 4E and F). Taz1 and Rap1 also interact with Bqt1 and Bqt2, which connect telomeres to the spindle pole body (SPB) upon entry into meiosis (31). However, telomeromic association of arm regions was not affected in sap1-1 bg11Δ and sap1-1 bg22Δ double mutants (Fig. S6G). Together, these results implicate Taz1–Shelterin in mediating new interactions between chromosome arm regions and telomeres in sap1-1 cells.

We also examined whether Shelterin components affect centromere–telomere contacts in sap1-1 cells. We found that in sap1-1 cells lacking Rap1, centromeres and telomeres remained associated with the nuclear periphery (Fig. S7A and B); however, the number of cells showing association between these loci decreased (Fig. S7C). Thus, in addition to facilitating connections between telomeres and chromosome arm regions, Shelterin components also seem necessary to mediate centromere–telomere contacts in sap1-1.

We wondered whether the genome reorganization observed in sap1-1 cells in response to replication stress is biologically relevant. Because certain types of DNA damage are targeted to nuclear compartments for specialized repair (32), we speculated that Shelterin-mediated association of arm regions with telomeres might affect the DNA damage-repair process. Indeed, the loss of Ccq1 or Rap1 in sap1-1 mutant cells resulted in a considerable increase in the number of Rad52 repair foci (Fig. S7D).

Discussion

The organization of eukaryotic genomes impacts many aspects of genome function, including replication and DNA-repair processes (2, 3). We find that cells carrying a mutation in Sap1 that show replication defects and genome instability undergo changes in genome organization. A remarkable finding is that components of the Shelterin telomere protection complex promote interactions between telomeres and specific chromosome arm regions. These results suggest an additional role for Shelterin in promoting genome reorganization with implications for understanding mechanisms that protect genome stability.

Sap1 has been suggested to play an important role in replication fork pausing at rDNA and retrotransposon LTRs (18, 20, 21). We show that Sap1 also facilitates replication progression, as indicated by 2D gel, Mcm6 localization, and BrdU incorporation analyses. In addition, replication defects are suggested by the accumulation of ssDNA and DNA repair foci in sap1-1 cells. Sap1 might impact replication through local chromatin changes, e.g., by affecting nucleosome occupancy (33). Another possibility is that Sap1, with its matrix-like nuclear localization, might serve as an architectural protein that binds and constrains chromosomes to promote their spatial positioning and proper replication. Such a role might be analogous to DNA-binding proteins in higher eukaryotes, such as CTCF, which recruits cohesin involved in genome organization (34). However, Sap1 is dispensable for cohesin-dependent globules. Instead, our preliminary analysis indicates that Sap1 copurifies with topoisomerase II (https://ccrod.cancer.gov/confluence/download/attachments/101483286/Sap1TopII.pdf?api=v2) implicated in replication and chromosome organization (35, 36). Regardless of its exact function, loss of Sap1 function affects proper replication, structural integrity, and organization of the genome.

sap1-1 cells show widespread genome reorganization, including association of arm regions with telomeres and the nuclear periphery. Because the affected arm regions contain replication origins and experience replication stress, it is conceivable that Sap1 indirectly affects genome organization through its impact on DNA replication/repair. Moreover, a low level of genomic rearrangements might contribute to the new interactions detected. To this end, we note that specific new interactions occur rapidly in cells that are cultured at a semipermissive temperature for only 6 h. Although these interactions potentially could lead to recombination events, whole-genome sequencing of sap1-1 did not reveal translocations between the newly interacting loci.

Our finding that Taz1–Shelterin mediates interactions between telomeres and arm regions has implications for understanding replication control and genome stability. Late firing of Taz1–affected origins requires telomere-associated Rif1, which also has been implicated in DNA repair (37, 38). However, Rif1 binds only a subset of Taz1–associated late origins (11, 38, 39), and it is conceivable that Shelterin-mediated telomeric association of these origins allows Rif1 acquisition to promote proper replication and DNA repair. In other words, Taz1-bound late origins in telomeric and arm regions might be controlled in a shared nuclear compartment. The physical proximity of regions experiencing replication stress to Rif1-enriched telomeres may also facilitate the resolution of DNA entanglements (40) and promote chromosome healing, in which telomerase “heals” dsDNA breaks (41). Finally, localization of these regions to the nuclear peripheral compartment may provide an opportunity to suppress and repair DNA damage, as observed in other systems (32, 42, 43). In this regard, we find that disruption of the telomeric association of arm regions in sap1-1 cells lacking Shelterin components correlates with increased accumulation of DNA damage. Moreover, certain Shelterin components show negative genetic interactions with DNA repair and checkpoint factors (44).

Collectively, these results link changes in genome organization to replication stress, which is an early driver of oncogenesis (45). Tandem duplication of chromosomal segments is a dominant class of structural change found in breast and ovarian cancers (46) and is thought to arise from the repair of replication stress-associated DNA breaks (47). Insights gained from S. pombe may aid studies in higher eukaryotes, particularly those focusing on the mechanisms underlying structural abnormalities and nuclear reorganization in replication-stressed cells.

Materials and Methods

WT and mutant strains were initially cultured in yeast extract adenine (YE)-rich medium at 26 °C and then were shifted to 33 °C for 6 h, unless otherwise
indicated. Growth conditions used to detect rearrangements, BrdU incorporation, and 2D gel analysis in sap1-1 cells are detailed in SI Materials and Methods. A description of Hc-C, 3C, ChIP-Chip, BrdU incorporation, nucleosome mapping, CGH, junction PCR, Southern blotting, 2D gel analysis, and FISH procedures can be found in SI Materials and Methods. Primers used in this study are listed in Table S2.

**Supporting Information**

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**SI Materials and Methods**

### Hi-C and 3C Analysis

The 3C and Hi-C libraries were generated as previously described using the restriction enzyme HindIII (8). 3C PCR was performed using the primers listed in Table S2. Hi-C data were mapped, and reads were filtered as described (8). Corrected contact probability matrices at 10-kb resolution were obtained using iterative correction (49). Both steps were performed using the hiclib library for python, publicly available at https://bitbucket.org/mirnylab/hiclib. Corrected Hi-C contact maps (Fig. S4 and Fig. S6A) and the rate of decay of intra-arm Pc values and the insulation plot (Fig. S6 B and C) were created as previously described (8).

### ChIP and ChIP-ChIP

ChIP was performed as previously described using anti-Sap1 antibody (rabbit polyclonal, a gift from B. Arcangioli, Dynamics of the Genome Unit, Department of Genomes and Genetics, Institut Pasteur, Paris), anti-Mcm6 (rabbit polyclonal, a gift from H. Masukata, Department of Biological Sciences, Graduate School of Science, Osaka University, Osaka), or anti-GFP (ab290; Abcam) for GFP-Bqt3 (50). Immunoprecipitated and input DNA were analyzed by quantitative duplex PCR using the primers listed in Table S2. Relative ChIP enrichment was determined as the ratio of the intensity of the duplex PCR product of the target to the control locus from ChIP samples normalized to an input DNA. Labeling, hybridization, and analysis of the microarray experiments were performed as described in ref. 12 using the genome-wide tiling microarray platform (50).

### Mcm6 ChIP Plot

HU was used to arrest WT and sap1-1 cells at S-phase. The log₂ ratio of Mcm6 ChIP enrichment in WT cells revealed distinct regions of enrichment across the genome. The Mcm6 signal was particularly enhanced at the subtelomeric regions and appeared as distinct peaks distributed across the genome. The 332 most prominent (nontelomeric) peaks on chromosome 1 were selected for specific comparison of Mcm6 binding in WT and sap1-1 cells. The heat map in Fig. 2C was generated using Java TreeView and shows the log₂ ratio (ChIP/INPUT) 10 kb on either side of the 332 identified peaks in WT and sap1-1 cells.

### BrdU Incorporation

DNA replication profiles were obtained by measuring the BrdU incorporation as previously described (12). The cdc10-v50 strains carrying the herpes simplex virus thymidine kinase expression module Pmnt1-TK and the human nucleoside transporter module PaudI-hENT were grown at 35 °C for 4 h and were released from the G1 block by transfer to medium supplemented with 200 μM BrdU and 10 mM HU at 26 °C for 90 min. BrdU-labeled DNA was extracted and used for immunoprecipitation with mouse anti-BrdU antibody (BD Pharmingen). Labeling, hybridization, and analysis of the microarray experiments were performed as for ChIP-chip experiments.

### Immunofluorescence and FISH

Immunofluorescence and FISH experiments were carried out as described (9, 23). Anti-Sap1 antibody (rabbit polyclonal, a gift from B. Arcangioli) and anti-GFP (ab290; Abcam) were used for immunofluorescence. The probe used for FISH analysis was prepared using TF2-ORF plasmid and pRS140 (centromeric repeat DNA) (23, 51). A position-specific probe for rnl1 was generated by long-range PCR using the primers listed in Table S2. Optical section data were collected using a DeltaVision Elite microscope (GE Healthcare) and were subsequently deconvolved using SoftWoRX 6.0 (GE Healthcare).

### Plate Assay for sap1-1 Revertant Isolation

WT and mutant cells cultured at 26 °C were spread onto rich YE agar plates and allowed to grow for 5 d. After colony formation, cells were replica plated and incubated at 37 °C for 7 d. Revertant colonies were counted and isolated for subsequent experiments.

### Liquid Culture for sap1-1 Revertant Isolation

Mutant strains were initially cultured at 26 °C. Rearrangements of wtf elements were detected in cells that were inoculated at a cell density of OD₆₀₀ ~0.02, shifted to 33 °C, and incubated for 3 d with periodic dilution to maintain a cell density of OD₆₀₀ 0.02-1.2. Isolated genomic DNA was used for junction PCR experiments to detect wtf-mediated tandem duplication.

### Junction PCR and Mapping the Recombination Junction

Genomic rearrangements mediated by wtf elements were detected by PCR using Takara Ex Taq HS (Takara Bio). The primers are listed in Table S2. PCR conditions used with wtf10-wtf8 and wtf10-wtf9 primer pairs were as follows: 94 °C for 1 min; 10 s, 98 °C for 3 min; and final elongation for 7 min. PCR conditions used with wtf18-wtf13 primer pairs were as follows: 94 °C for 1 min; 30 s, 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 4 min with a final elongation for 7 min. Junction PCR products were subjected to direct DNA sequencing to map the recombination junction.

### Array CGH

Genomic DNA from WT or sap1-1 revertants was digested with AluI and Rsal. After complete digestion, DNA from WT and sap1-1 revertants was labeled with Cy-3 dCTP and Cy-5 dCTP, respectively (Amersham Biosciences) using the BioPrime Array CGH Genomic Labeling kit (Invitrogen). Equal amounts of labeled DNA (1.5 μg) were competitively hybridized onto the genome-wide tiling microarray platform (50). Prehybridization, probe hybridization, washing, and drying steps for arrays were performed as for ChIP-chip experiments. The log₂-transformed Cy5/Cy3 ratio is plotted along the chromosome.

### Southern Analysis

Genomic DNA was digested with Apal and DraI for analyzing the subtelomeric repeat and sap1-1 gene amplification, respectively. The subtelomeric repeat sequence probe was made by digesting pAMP002 with Apal + EcoRI. Probes for sap1 and ade6 were generated by PCR using the primers listed in Table S2.

### 2D Gel Analysis

cdc10-v50 and cdc10-v50 sap1-1 cells were grown in G1 by incubation at 35 °C for 4 h. After a shift to 25 °C to resume DNA synthesis, 250 mL (OD₆₀₀ ~0.5) were harvested following the addition of 50 mL 200 mM EDTA and 2.5 mL 10% NaN₃. Cells were washed in CSE buffer (1.2 M sorbitol, 20 mM citrate phosphate, 40 mM EDTA), and the cell wall was digested using 0.25 mg/mL Zymolyase 100T (Seikagaku) in CSE buffer for 30 min. Cells were pelleted and resuspended in
CSE buffer. An equal amount of 1% InCert agarose (Cambrex) was added, and cells were embedded into an agarose plug mold, which was digested in solution (10 mM Tris, 1% lauryl sarcosine, 25 mM EDTA, 1 mg/mL Proteinase K) overnight at 50 °C. The digestion solution was removed, followed by incubation in storage buffer (10 mM Tris, 50 mM EDTA) twice for 1 h. DNA agarose plugs were washed three times in Tris/EDTA (TE) buffer and twice in restriction enzyme buffer. DNA plugs were incubated with an appropriate restriction enzyme (40 U per plug) at 37 °C overnight. Agarose was melted by heating to 65 °C for 5 min and to 37 °C for 5 min. An additional 40 U of restriction enzyme was added for 2 h at 37 °C. Digested DNA was separated on a 0.4% agarose gel in Tris/borate/EDTA (TBE)+3 mM MgCl₂ buffer. The first dimension was run at 1.2 V/cm for 21 h at room temperature. The second dimension was run at 6 V/cm for 5 h with buffer circulation at 4 °C through a 0.9% agarose gel in TBE + 3 mM MgCl₂ buffer containing 0.4 μg/mL ethidium bromide. Southern transfer and hybridization were carried out using a position-specific wtf9 probe generated by PCR using the primers listed in Table S2.

![Image of Sap1 ChIP enrichment](image)

**Fig. S1.** Sap1 is an abundant nuclear DNA-binding protein and localizes broadly across the genome. (A) Subcellular localization of Sap1. Sap1 localizes to the nucleus, whereas the mutant Sap1-1 is diffused throughout the cell. (B) The total protein levels of Sap1 and Sap1-1 were determined by Western blot (WB) using anti-Sap1 antibody. TAT1 was used as a loading control. (C) ChIP-chip analysis of Sap1 and Sap1-1 protein. Black, green, and red circles indicate the genomic positions of LTR, wtf, and Tf2 elements, respectively. (D) ChIP-sequencing analysis of Sap1 [adapted from Zaratiegui et al. (18)] compared with nucleosome occupancy. MNase hypersensitivity mapping data are adapted from our previous study (52). Green bars represent ORFs according to the 2007 *S. pombe* genome assembly.
Fig. S2. sap1-1 causes a shift in chromosome configuration and allows new genomic contacts to be made with the nuclear membrane. (A) Genome-wide GFP-Bqt3 ChIP-chip analysis of WT and sap1-1 cells. (B, Left) GFP-Bqt3 ChIP enrichment at centromere 2. The vertical line indicates individual copies of tRNAs; the red box indicates the central core domain (cnt); the gray arrows indicate the innermost repeats (imr); the blue arrows indicate the outer repeat region (otr). (Right) GFP-Bqt3 ChIP enrichment at subtelomeric repeats. (C) GFP-Bqt3 ChIP enrichment across extended subtelomeric domains and certain chromosome arm regions where new genomic contacts with the nuclear membrane emerge in sap1-1 cells. Orange and blue circles represent early- and late-replication origins, respectively, as annotated by Hayashi and colleagues (48).
Fig. S3. Replication profiles of WT and sap1-1 cells. (A) Replication profile of a segment of chromosome 1 in WT cells. Detection of early-replication origins in the cdc10-v50 single-mutant background by BrdU immunoprecipitation. Cells were released from the G1-phase in the presence of HU. (B) Replication profiles of subtelomeric regions in WT (Top) and sap1-1 (Middle) cells. Cells carrying the cdc10-v50 allele were arrested and released from G1-phase in the presence of HU. (Bottom) The difference between sap1-1 and WT cells is plotted. Orange and blue circles represent the early- and late-replication origins, respectively, as annotated by Hayashi and colleagues (48). (C) Mcm6 ChIP-chip analysis of chromosome 1 in WT and sap1-1 cells. (D) DNA replication profile of chromosome arm regions in WT and sap1-1 cells. GFP-Bqt3 and Mcm6 ChIP-chip are shown in the top and bottom graphs, respectively. IP, immunoprecipitation.
Fig. S4. Replication-associated DNA damage followed by division may cause chromosome fragmentation in sap1-1 cells. (A) The RPA component Rad11-GFP forms discrete foci in sap1-1 cells. Cells expressing Rad11-GFP (green) and Hht1-RFP (red) were cultured at 26 °C and then were shifted to 33 °C for 6 h. (Scale bar, 5 μm.) (B) Time-lapse observation of Rad11-GFP (green) and Hht1-RFP (red) in sap1-1 cells during M-phase. Projection images of each indicated time point are shown. Numbers below the images indicate the time in minutes. Chromatin (Hht1-RFP) appears fragmented and lags behind during M-phase in sap1-1 cells (arrowheads). Rad11-GFP is observed near fragmented chromatin masses (arrow). (Scale bars, 5 μm.) (C) Rad52 forms nuclear foci in sap1-1 cells. Cells expressing Rad52-YFP were cultured at 26 °C and then were shifted to 33 °C for 6 h. The percentage of mononuclear cells with Rad52-YFP foci (n = 84 for WT cells and n = 103 for sap1-1 cells) is shown. (Scale bar, 5 μm.) (D) Fivefold serial dilution assay of indicated strains on complete medium at 30 °C.

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Fig. S5. Repeat structures, including wtf, are destabilized in sap1-1 cells. (A) Microarray CGH analysis of revertants reveals a copy number gain of a region encompassing the sap1-1 locus on chromosome 3 (arrow). (B) The genomic alteration was confirmed by Southern blot analysis. A mixture of sap1 and ade6 probes was used for southern hybridization. Both sap1 and ade6 genes map to chromosome 3, but ade6 is located outside of the copy number gain region. The sap1-1 signal is higher in the revertants than in the parental sap1-1 strain. The ade6 locus was used as a loading control. (Lower) Western blot analysis of whole-cell lysates prepared from sap1-1 cells and a revertant with a sap1-1 duplication. The duplication results in the increased total protein level of Sap1-1. TAT1 was used as a loading control. (C) Junction PCR was used to determine that the copy number gain results from direct tandem-oriented duplication. All revertants contained a common copy number gain ∼100- to 150-kb in size. (D) Rearrangements are mediated by the 47- to 440-bp identical DNA sequence in wtf elements (highlighted in green). Junction PCR products were subjected to Sanger DNA sequencing to map the recombination junction. (E) Southern blot analysis of ApaI-digested genomic DNA using the subtelomeric repeat sequence probe. sap1-1 cells were cultured at the semipermissive temperature, and genomic DNA was isolated from independent colonies (derivatives). (F) Junction PCR analysis of wtf-mediated recombination in a strain lacking the HR factor Rad52. (G) Junction PCR analysis of wtf-mediated recombination in a strain expressing mutant Clr3 and Clr6 HDAC proteins (clr3-735 clr6-1), which have been implicated in the repression of wtf.
Fig. S6. Fundamental features of *S. pombe* genome organization are preserved in *sap1-1* cells. (A) Genome-wide Hi-C heatmaps for WT and *sap1-1* cells at 10-kb resolution. WT data are adapted from Mizuguchi et al. (8). (B) Contact probability as a function of genomic distance for different chromosomal arms. The decay of intra-arm contact probability as a function of genomic distance, plotted for each chromosome arm. Contact probability $P(s)$ values decrease more slowly at short distances. The black and gray dashed lines represent the slope for fractal globules ($-1$) and polymers in a melt ($-3/2$), respectively. (C) Relative probability of contact around a cohesin peak as a function of insulation distance averaged over all cohesin peaks (insulation plot). Contact frequency between regions separated by cohesin peaks was depleted in both WT and *sap1-1* cells. (D) Cohesin (Psc3-GFP) ChIP-chip data of a segment of chromosome 2 in WT and *sap1-1* cells. The binding profile of cohesin is unaffected in *sap1-1* cells. (E) The regions of chromosome arms in which new interactions emerge in *sap1-1* cells. Distances from the center of the new Hi-C interaction region to the nearest Taz1 ChIP enrichment are shown. (F) Genomic DNA isolated from WT and *sap1-1* cells was analyzed using the 3C PCR primer. WT and mutant strains were initially cultured at 26 °C and then were shifted to 33 °C for 6 h. (G) 3C PCR analysis of a *sap1-1*-specific interaction (chr.1: 4,590-kb region and subtelomere 1R) in *sap1-1 bqt1Δ* and *sap1-1 bqt2Δ* cells.
Fig. S7. Shelterin facilitates specific interactions in sap1-mutant cells. (A) Nuclear peripheral localization of centromeres in WT and sap1-1 cells. The shortest 2D distance between the centromere FISH signal (pRS140) and the NE (GFP-Bqt4) in the focal plane near the nuclear midplane was measured and classified as zone I (nuclear periphery, 0–0.22 μm from the NE), zone II (middle, 0.23–0.51 μm from the NE), or zone III (nuclear interior, 0.52–1.20 μm from the NE). (B) Nuclear peripheral localization of Taz1-YFP (telomeres) in WT and sap1-1 cells. The shortest 2D distance between Taz1-YFP and the NE (GFP-Bqt4) in the focal plane near the nuclear midplane was measured and classified into three zones (n = 94 for WT and n = 166 for sap1-1 cells). The dashed line at 33% corresponds to random distribution. (C) Graph of the percentage of mononuclear cells showing colocalization of telomeres (Taz1-GFP) and SPB (Sad1-mRFP). WT and mutant strains were initially cultured at 26 °C and then were shifted to 33 °C for 6 h. ****P ≤ 0.0001 (two-tailed Mann–Whitney test). Two independent experiments; total n = 207 (26 °C, WT), n = 299 (33 °C, WT), n = 198 (26 °C, sap1-1), n = 207 (33 °C, sap1-1), n = 328 (26 °C, sap1-1 rap1Δ), n = 288 (33 °C, sap1-1 rap1Δ). (Left) Time-lapse images were recorded at 30-s intervals for 3 min. Associations were determined by codirectional movement and colocalization. Representative images of indicated strains are shown. Arrows indicate telomere–SPB association. (D) Cells expressing Rad52-YFP were cultured at 26 °C and then were shifted to 33 °C for 6 h. The percentage of mononuclear cells with Rad52-YFP foci is shown. n, total number of cells examined for each strain. (Scale bars, 5 μm in A–C.)
Table S1. Correlation of Bqt3-enriched sites with DNA replication origins in *sap1-1* cells

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The 2007 *S. pombe* genome assembly was used for start and end coordinates of regions. E, Early origin; L, late origin; RO, replication origin.
Table S2. Primers used in this study

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Movie S1. Live-cell imaging of Rad11 foci in sap1-1 cells through mitosis (example 1). Time-lapse microscopy of a representative cell shows chromosomes during M-phase as revealed by the red RFP-tagged histone, Hht1-RFP. A discrete Rad11-GFP green focus is observed near the fragmented chromatin masses in sap1-1. Cells were imaged on a DeltaVision Elite microscope (GE Healthcare) with a 100× 1.4 NA Plan Super Apochromat oil lens (Olympus) using SoftWoRX 6.0.
**Movie S2.** Live-cell imaging of Rad11 foci in sap1-1 cells through mitosis (example 2). Time-lapse microscopy showing chromosomes marked by red RFP-tagged histone, Hht1-RFP, during M-phase. Cells were imaged on a DeltaVision Elite microscope (GE Healthcare) with a 100× 1.4 NA Plan Super Apochromat oil lens (Olympus) using SoftWoRX 6.0.

**Movie S3.** Centromere and telomere localization in sap1-1 cells (example 1). Time-lapse microscopy of a representative cell reveals continual overlap of centromeres (marked by red Sad1-RFP) and telomeres (marked by green Taz1-GFP) in sap1-1. Cells were imaged on a DeltaVision Elite microscope (GE Healthcare) with a 100× 1.4 NA Plan Super Apochromat oil lens (Olympus) using SoftWoRX 6.0.
Movie S4. Centromere and telomere localization in sap1-1 cells (example 2). Time-lapse microscopy of a sap1-1 cell showing continual overlap of Sad1-RFP (centromeres) and Taz1-GFP (telomeres). Cells were imaged on a DeltaVision Elite microscope (GE Healthcare) with a 100× 1.4 NA Plan Super Apochromat oil lens (Olympus) using SoftWoRX 6.0.

Movie S5. Centromere and telomere localization in WT cells (example 1). Time-lapse microscopy of a representative WT cell, in which continual overlap of centromeres (marked by red Sad1-RFP) and telomeres (marked by green Taz1-GFP) is not observed. Cells were imaged on a DeltaVision Elite microscope (GE Healthcare) with a 100× 1.4NA Plan Super Apochromat oil lens (Olympus) using SoftWoRX 6.0.
Movie S6. Centromere and telomere localization in WT cells (example 2). Time-lapse microscopy showing a lack of any continual overlap of Sad1-RFP–marked centromeres and Taz1-GFP telomeres. Cells were imaged on a DeltaVision Elite microscope (GE Healthcare) with a 100× 1.4 NA Plan Super Apochromat oil lens (Olympus) using SoftWoRx 6.0.

Movie S6