LETTER

doi:10.1038/nature09608

# CENP-B preserves genome integrity at replication forks paused by retrotransposon LTR

Mikel Zaratiegui[1], Matthew W. Vaughn[1]†, Danielle V. Irvine[1]†, Derek Goto[1]†, Stephen Watt[2]†, Jürg Bähler[2], Benoit Arcangioli[3] & Robert A. Martienssen[1]

**Centromere-binding protein B (CENP-B) is a widely conserved DNA binding factor associated with heterochromatin and centromeric satellite repeats[1]. In fission yeast, CENP-B homologues have been shown to silence long terminal repeat (LTR) retrotransposons by recruiting histone deacetylases[2]. However, CENP-B factors also have unexplained roles in DNA replication[3,4]. Here we show that a molecular function of CENP-B is to promote replication-fork progression through the LTR. Mutants have increased genomic instability caused by replication-fork blockage that depends on the DNA binding factor switch-activating protein 1 (Sap1), which is directly recruited by the LTR. The loss of Sap1-dependent barrier activity allows the unhindered progression of the replication fork, but results in rearrangements deleterious to the retrotransposon. We conclude that retrotransposons influence replication polarity through recruitment of Sap1 and transposition near replication-fork blocks, whereas CENP-B counteracts this activity and promotes fork stability. Our results may account for the role of LTR in fragile sites, and for the association of CENP-B with pericentromeric heterochromatin and tandem satellite repeats.**

In fission yeast, CENP-B proteins are encoded by three homologues, autonomously replicating sequence binding protein 1 (*abp1*), cenp-B homologue 1 (*cbh1*) and *cbh2*, and were previously characterized as DNA binding factors at origins of replication and centromeric repeats, respectively[3,5]. Mutants of *abp1* grow slowly, whereas double mutants with *cbh1* or *cbh2* have severely stunted growth, abnormal mitosis and morphological defects, and triple deletion mutants are inviable[6,7]. As a result, double *Δabp1Δcbh1* mutants form microcolonies on solid media (Fig. 1a and Supplementary Table 1) and exhibit high levels of cell death (Supplementary Fig. 1). We observed the spontaneous appearance of faster growing cells in a culture of *Δabp1Δcbh1* that grew at rates similar to the *Δabp1* single mutant, lacked morphological defects (Fig. 1a and Supplementary Table 1) and showed lower levels of cell death (Supplementary Fig. 1). Genetic analysis revealed the presence of a single essential locus that also suppressed the lethality of the triple mutant *Δabp1Δcbh1Δcbh2* (not shown). We performed whole-genome resequencing in the mutant strain[8] and isolated a missense mutation in the coding sequence of the DNA binding factor Sap1 (*sap1E101D*, henceforth called *sap1-c*; Supplementary Fig. 2) that co-segregated with suppression of slow growth in *Δabp1Δcbh1* and resulted in lethality in a wild-type background. Sap1 is a protein with essential roles in chromosome stability[9]. Sap1 has been implicated in a programmed replication-fork block in the ribosomal DNA (rDNA) monomer that ensures directional replication to prevent mitotic recombination between rDNA repeats[10–12].

To test the effects of CENP-B and *sap1-c* mutations on genome integrity, we examined chromosomes by pulsed-field gel electrophoresis. Although single *Δabp1* and *Δcbh1* mutants had wild-type chromosome lengths, the double *Δabp1Δcbh1* mutant had a smear of DNA fragments indicating double-strand breaks in all three chromosomes (Fig. 1b). Treatment of the *Δabp1Δcbh1* sample plugs with the restriction enzyme NotI allowed migration of the chromosomes into the gel, and detection of telomeric and centromeric sequences (Supplementary Fig. 3), suggesting the presence of scattered unresolved replication or recombination intermediates that interfere with the migration of full-length chromosomes,

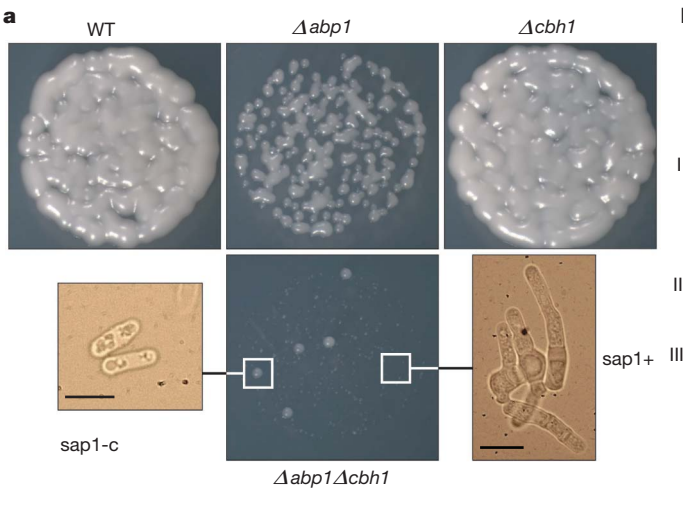


**Figure 1 | DNA damage in CENP-B mutants is suppressed by *sap1* mutation. a**, Images of $10^3$ plated cells of wild type (WT), *Δabp1*, *Δcbh1* and *Δabp1Δcbh1* with *Δabp1Δcbh1sap1-c* colonies. Microscopy image inserts: branched phenotype in *Δabp1Δcbh1* background (right) and *Δabp1Δcbh1sap1-c* mutant (left). Scale bar, 10 μm. **b**, Pulsed-field gel blot analysis of WT, CENP-B mutants (*Δabp1*, *Δcbh1*, *Δabp1Δcbh1*) and five CENP-B/*sap1-c* mutant isolates (*Δabp1Δcbh1sap1-c*). The position of the three chromosomes is indicated on the left. The image is a false-coloured composite of hybridizations for all three chromosomes.

[1]Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, New York 11724, USA. [2]University College London, Department of Genetics, Evolution & Environment, and UCL Cancer Institute, Darwin Building, Gower Street, London WC1E 6BT, UK. [3]Institut Pasteur, Dynamics of the Genome Unit, Departments of Genomes and Genetics and Developmental Biology, CNRS URA2171, F-75015 Paris, France. †Present addresses: Texas Advanced Computing Center, University of Texas at Austin, 10100 Burnet Road, Austin, Texas 78758, USA (M.W.V.); Chromosome and Chromatin Research, Murdoch Childrens Research Institute, Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Flemington Road, Parkville 3052, Australia (D.V.I.); Creative Research Initiative Sousei, Hokkaido University. North-21, West-10, Kita-ku, Sapporo, 001-0021, JAPAN (D.G.); Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Cambridge CB2 0RE, UK (S.W.).

but not with NotI-digested DNA, into the pulsed field gel. This indicates that Abp1 and Cbh1 have roles in the maintenance of genome integrity.

Surprisingly, the *sap1-c* mutation restored genome integrity to all chromosomes, with chromosome 3 exhibiting size variability in several isolates of *Δabp1Δcbh1sap1-c* mutant (Fig. 1b). In fission yeast, chromosome 3 harbours the rDNA repeats. Temperature-sensitive alleles of *sap1* exhibit changes in the size of chromosome 3 attributable to loss of fork barrier activity and an increase in mitotic recombination at rDNA[12]. The changes in the size of chromosome 3 size in the *sap1-c* mutants are associated with altered rDNA copy number (Supplementary Fig. 4). The temperature-sensitive alleles *sap1-1* and *sap1-48* (ref. 12) suppressed slow growth in the *Δabp1Δcbh1* double mutant, mimicking *sap1-c* (Supplementary Fig. 5). Consistent with a reduction in fork barrier activity, a probe containing a canonical Sap1-binding sequence had reduced electrophoretic mobility shift in crude extracts from *sap1-c* mutants (Supplementary Fig. 6). We conclude that the suppression of the *Δcbh1Δabp1* phenotype is not specific to the *sap1-c* mutation but a result of defective function of Sap1, and therefore that the loss of genome integrity in *Δabp1Δcbh1* mutants is a consequence of Sap1 activity.

Blocked replication forks are potential sources of genome instability because they can lead to collapse of the replisome and double-strand break formation[13]. The fact that Sap1 activity leads to DNA damage in the absence of Abp1/Cbh1 suggests that the function of CENP-B is to manage Sap1-arrested replication forks. In the absence of Sap1, loss of replication-fork blockage would render Abp1/Cbh1 activity unnecessary and lead to increased genome stability in *Δabp1Δcbh1* mutants. This model predicts that CENP-B and Sap1 would co-localize to the regions where they acted on the replication fork, and that these regions would engage in homologous recombination and degrade to double-strand breaks in the absence of CENP-B. To test this hypothesis, we performed chromatin immunoprecipitation of Sap1, Abp1 and Cbh1 followed by high-throughput sequencing (ChIP-seq). Abp1 has previously been shown to localize and recruit Cbh1 to the LTRs of Tf1 and Tf2 retrotransposons, where Abp1/Cbh1 play a role in their transcriptional silencing[2]. We demonstrated a strong co-localization of Sap1 with Abp1 and Cbh1 at these LTRs as well as at solo LTRs scattered throughout the genome (Fig. 2a, c and Supplementary Fig. 7a, b) and at the mating type locus (Supplementary Fig. 8), where Sap1 and Abp1 have been described to regulate mating-type switching[14,15]. Both Sap1 and Abp1/Cbh1 also localized to genomic regions independently of each other, suggesting that they do not form a stable complex or mediate their mutual recruitment. In particular, Abp1 exhibited binding to transfer RNA (tRNA) genes (Fig. 2b and Supplementary Fig. 7b), known to be potent replication pause sites[13,16]. Abp1 and Cbh1 co-localize to a highly A/T-rich region located in positions 100–150 of the LTR (Fig. 2c and Supplementary Fig. 7a, b). The localization of Sap1 within the LTR was concentrated in the first 50 base pairs of sequence (Fig. 2c), coinciding with a predicted Sap1-binding site[17] (Supplementary Fig. 7a, c). We tested this sequence by electrophoretic mobility shift assay and detected specific binding in wild-type extracts (Fig. 2d) as well as decreased binding and altered mobility in extracts from *Δabp1Δcbh1sap1-c* mutants (Supplementary Fig. 7d). Interestingly, solo LTR and full-length Tf2 insertions were associated with a prominent peak of Sap1 binding located outside the 3′ end of the transposon sequence (Fig. 2c). These observations indicate that Sap1 binding precedes and possibly guides Tf element integration. To test this prediction, we plotted the average enrichment of Sap1, Abp1 and Cbh1 around more than 70,000 *de novo* Tf1 integration sites recently reported[18]. We observed a dramatic association of these integration sites with a peak of Sap1 binding immediately downstream of the insertion site (Fig. 2e and Supplementary Fig. 8) and no appreciable CENP-B enrichment. These results strongly suggest that Sap1-binding sequences determine the targeting and orientation of Tf retroelement transposition.

To evaluate the mutual influence of Sap1 and Abp1/Cbh1 on LTR binding, we performed ChIP analysis of Sap1 in *Δabp1Δcbh1* mutants and of Abp1 in temperature-sensitive *sap1* mutants that affect DNA-binding activity[12]. Sap1 binding to the LTR was unaffected in *Δabp1* and *Δcbh1* mutants, and was slightly increased in *Δabp1Δcbh1* double mutants (Fig. 2f), but consistently reduced (twofold) in *Δabp1Δcbh1sap1-c*. Conversely, Abp1 binding to the LTR was increased between two and three times at the permissive temperature in *sap1-1* and *sap1-48* mutants (Fig. 2g). These results indicate that Sap1 and Abp1/Cbh1 bind to the LTR independently of each other and mutually counteract their recruitment, and that the *sap1-c* mutation impairs its binding to the LTR *in vivo* as well as *in vitro* (Supplementary Fig. 4).

A failure of replication-fork stability at LTRs, which are distributed throughout the genome, would explain the widespread DNA damage in *Δabp1Δcbh1* mutants. We assessed the behaviour of the replication

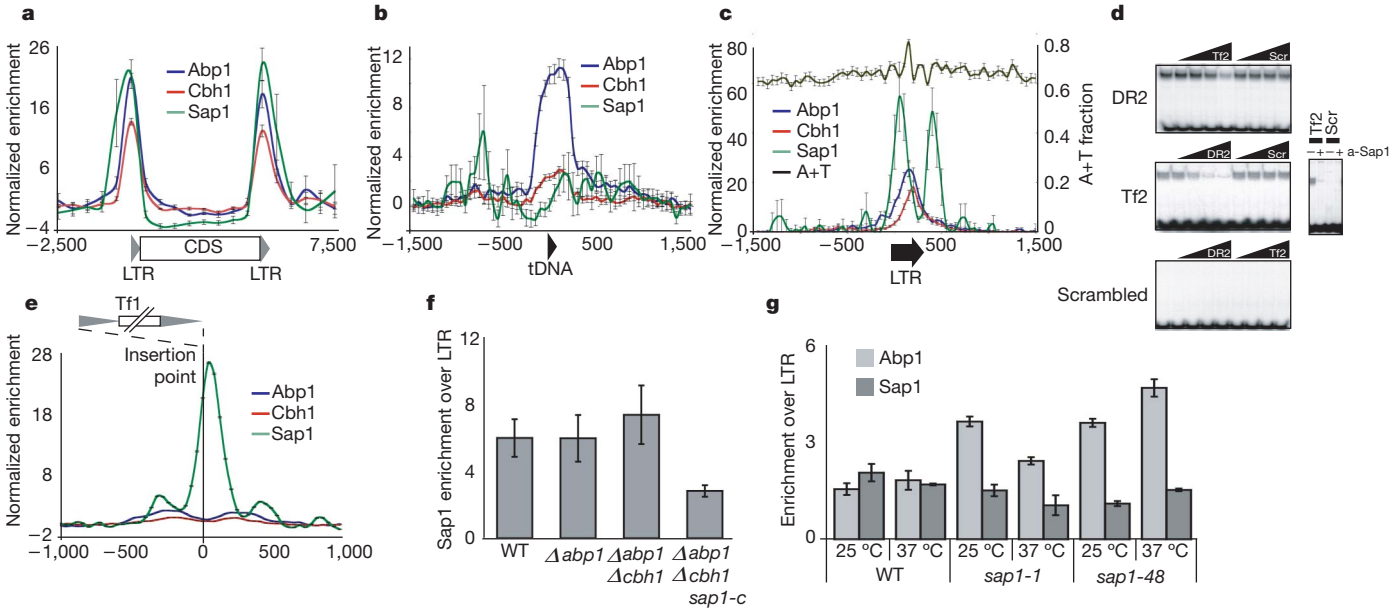


**Figure 2 | Sap1 and CENP-B co-localize at the LTR of retrotransposons *in vivo*.** Average genome-wide enrichment by ChIP-seq of Sap1, Abp1 and Cbh1 on (**a**) all *Tf2* elements, (**b**) euchromatic tRNA and (**c**) solo LTR. Error bars, s.e.m. **d**, Left panel, competition electrophoretic mobility shift assay; right panel, inactivation by incubation with anti-Sap1 serum[9]. **e**, Average Sap1, Abp1 and Cbh1 enrichment around Tf1 *de novo* insertion points[18]. **f**, ChIP of Sap1 with LTR of *Tf2* in CENP-B and *sap1-c* mutants and (**g**) of Abp1 with LTR of *Tf2* in *sap1* temperature-sensitive mutants. Error bars, s.d. for triplicates.

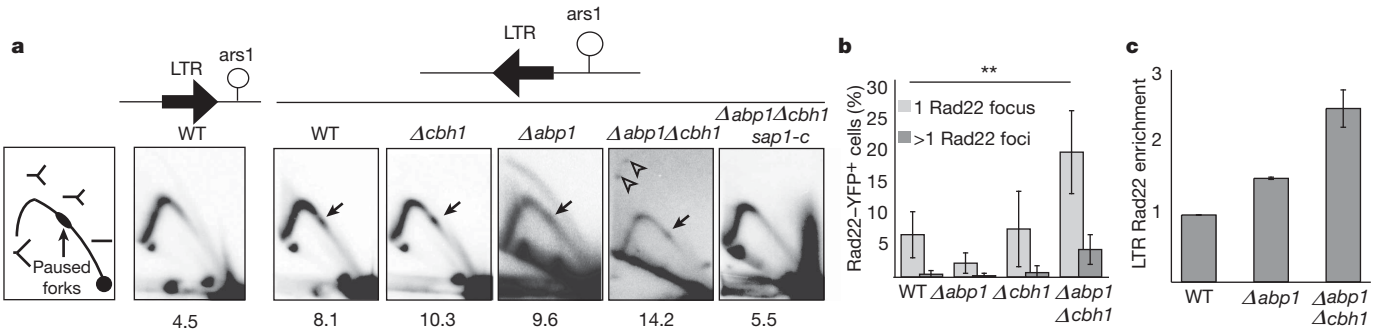


**Figure 3 | CENP-B promotes replication-fork progression through the Sap1-dependent barrier present at the LTR and prevents homologous recombination.** **a**, Two-dimensional gel electrophoresis of a plasmid fragment containing the *Tf2* LTR oriented towards (left) and away (right) from the *ars1* origin. Arrows, paused replication intermediates; open arrows, recombination intermediates. The percentage of signal over the LTR is indicated below each panel. **b**, Quantification of Rad22–YFP foci ($n > 400$ nuclei for all mutants). Error bars, s.e.m. **c**, Rad22–YFP ChIP with LTR in wild type, *Δabp1* and *Δabp1Δcbh1* mutants. Error bars, s.d. for triplicates.

fork as it traversed the LTR using two-dimensional agarose gel electrophoresis. Sap1-dependent programmed fork blocks are directional and only hinder fork progression in one orientation[10,11]. We cloned a full-length LTR and its first 50 base pairs (containing the Sap1-binding site) in a plasmid in both orientations with respect to the replication origin *ars1*. Two-dimensional gel electrophoresis in a wild-type strain transformed with this episomal system showed a modest accumulation of fork signal at the location of the cloned LTR (Fig. 3a), but only when the Sap1-binding site was proximal to the origin, and not in the opposite orientation (Supplementary Fig. 9). The Sap1-binding site was sufficient for this blocking activity, with the same orientation requirement (Supplementary Fig. 9). We next assayed the LTR for pausing activity in *Δabp1*, *Δcbh1* and *sap1-c* mutants (Fig. 3a). Strikingly, the paused fork signal was consistently enhanced and always at the same location in *Δabp1* and *Δcbh1* mutants, whereas the *Δabp1Δcbh1* double mutant exhibited additional signals outside the replication arc, suggestive of recombination intermediates[19]. The fork-blocking activity of the LTR disappeared in *Δabp1Δcbh1sap1-c* mutants. Unresolved fork blocks can collapse and undergo homologous recombination for fork recovery. We confirmed the presence of homologous recombination in the *Δabp1Δcbh1* double mutants by measuring the increase in the formation of Rad22 (homologous to Rad52 in *Saccharomyces cerevisiae*) foci in a Rad22–yellow fluorescent protein (YFP) strain[20] (Fig. 3b). We observed that *Δabp1Δcbh1* double-mutant cells accumulated the homologous recombination protein Rad22 at the LTR (Fig. 3c). Consistently, the recombination factor Rhp51 (Rad51 homologue) was essential for viability of *Δabp1Δcbh1* double mutants (Supplementary Fig. 10), indicating that homologous recombination is necessary for recovery from fork stalling at LTRs. These results indicate that Abp1/Cbh1 counteract Sap1 barrier activity and stabilize the replication fork at LTRs. This results in loss of genome integrity and homologous recombination at the LTR in *Δabp1Δcbh1* mutants.

The Sap1-binding sequence is conserved in Tf1 and Tf2 retrotransposon LTRs (Supplementary Fig. 7c), suggesting that it plays a role in the retrotransposon life cycle. We assayed the effect of *sap1* and *abp1/cbh1* on Tf2 stability by measuring the frequencies of loss of a *ura4* reporter transgene inserted in the Tf2-6 transposon[21]. Mutation of *abp1* resulted in a dramatic decrease of Tf2 ectopic recombination, which returned to normal levels when *sap1* was also mutated (Fig. 4). In the presence of *sap1+* there is a preference for gene conversion, which normally constitutes most ectopic recombination events[22]; however, in *Δabp1sap1-c* and *Δcbh1sap1-c* mutants the proportion of eviction and conversion events is similar (Fig. 4). Therefore we propose that the LTR recruits Sap1 to control the direction of transposon replication and increase transposon persistence in the genome, perhaps by coordinating lagging strand synthesis, which prevents single-strand annealing from complementary direct repeats (Supplementary Fig. 11a, b). CENP-B counteracts this activity, possibly by promoting replication-fork progression through the Sap1-dependent barrier. Thus CENP-B and Sap1 promote genome and transposon integrity, respectively, in a 'tug-of-war' between transposon and host. Abp1 stimulates fork progression by recruiting the

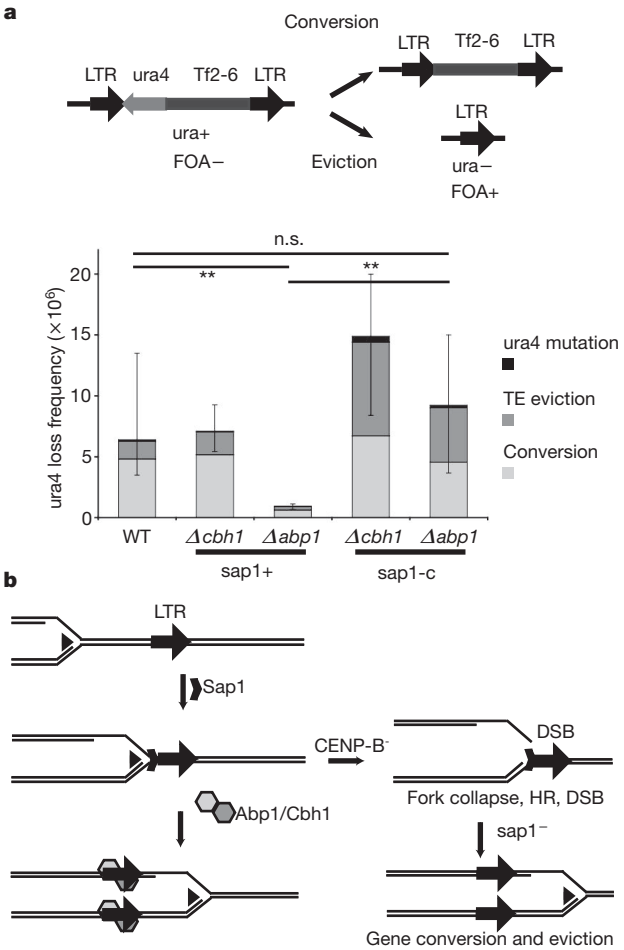


**Figure 4 | CENP-B and Sap1 have opposite effects on Tf2 stability.** **a**, Ectopic recombination fluctuation assay. Two potential mechanisms of *ura4* loss from the marked *Tf2-6::ura4* are indicated: gene conversion and eviction by LTR recombination. Columns represent total median *ura4* loss frequency in wild type, *Δabp1*, *Δcbh1*, *Δabp1sap1-c* and *Δcbh1sap1-c* mutants; error bars, 95% confidence intervals. Tints indicate distribution of mode of ectopic recombination events in the *ura4*$^-$ colonies obtained from wild type ($n = 93$), *Δabp1* ($n = 88$), *Δcbh1* ($n = 94$), *Δabp1sap1-c* ($n = 91$) and *Δcbh1sap1-c* ($n = 89$) mutants. **b**, Model for the interactions between Abp1, Cbh1, Sap1 and the replication fork at the LTR.

fork-restart protein MCM10 (ref. 4), which has primase activity. Additionally the histone deacetylase Mst1, which has roles in replication-fork stability interacts directly with Cbh1 (ref. 23). In *S. cerevisiae* the histone deacetylase Sir2 silences and inhibits recombination in repetitive DNA[24]. CENP-B factors recruit the histone deacetylases Clr3 and Clr6, which perform LTR silencing[2]. The result of these functions would be to preserve genome integrity at LTRs by preventing DNA damage and recombination. This novel role of CENP-B may not be limited to LTR and tDNA, as mutation of the replication-fork blocking factor *reb1*, which is specific to rDNA repeats, also suppresses the slow growth of the *abp1* mutant[25]. Similarly, our ChIP-seq data indicate that Sap1 may also be implicated in the functionality of the replication terminator RTS1 (Supplementary Fig. 8) in collaboration with Rtf1. In this manner, the function and regulation of the Sap1-bound regions is determined by the binding in their vicinity of different factors affecting replication-fork progression.

Because of their repetitive nature, transposons have a close relationship with replication and recombination. For example, the IS608 transposon of *Escherichia coli* is targeted to the lagging strand and always replicated in the same direction[26]. This might prevent recombination between tandemly arranged copies. We have shown that retrotransposons influence DNA replication through recruitment of directional fork blocking factor Sap1 and that activity of CENP-B is required for replication-fork management. Additionally, retrotransposition is targeted to the genomic localization of Sap1. These mechanisms influence the replicative dynamics of the host genome. The genomes of eukaryotes show widespread colonization by retrotransposons, and pericentromeric satellite repeats are often of transposon origin[27]. When such sequences are arranged as tandem repeats, control of replication direction by CENP-B would prevent chromosome breaks and preserve genome integrity. This mechanism accounts for the role of other regulators of fork progression in inter-LTR recombination[28,29]. In contrast, when flanked by LTR in opposite orientations, fragile sites fail to replicate and result in chromosome breaks[13,30].

## METHODS SUMMARY

ChIP was performed using tagged TAP-Abp1 and TAP-Cbh1 strains with an Anti-Calmodulin Binding Protein antibody (Millipore) and a polyclonal serum against the native Sap1 protein[9]. High-throughput sequencing was performed on an Illumina G2 genome analyser, and analysed for polymorphism detection or statistical analysis of enrichment. Two-dimensional gel electrophoresis was performed as described[11]; see Supplementary Information for construction of the episomal system. Electrophoretic mobility shift assay was performed as described previously[17].

**Received 15 July; accepted 22 October 2010.**
**Published online 12 December 2010.**

1. Okada, T. *et al.* CENP-B controls centromere formation depending on the chromatin context. *Cell* **131,** 1287–1300 (2007).
2. Cam, H. P., Noma, K., Ebina, H., Levin, H. L. & Grewal, S. I. Host genome surveillance for retrotransposons by transposon-derived proteins. *Nature* **451,** 431–436 (2008).
3. Murakami, Y., Huberman, J. A. & Hurwitz, J. Identification, purification, and molecular cloning of autonomously replicating sequence-binding protein 1 from fission yeast *Schizosaccharomyces pombe*. *Proc. Natl Acad. Sci. USA* **93,** 502–507 (1996).
4. Locovei, A. M., Spiga, M. G., Tanaka, K., Murakami, Y. & D'Urso, G. The CENP-B homolog, Abp1, interacts with the initiation protein Cdc23 (MCM10) and is required for efficient DNA replication in fission yeast. *Cell Div.* **1,** 27 (2006).
5. Lee, J. K., Huberman, J. A. & Hurwitz, J. Purification and characterization of a CENP-B homologue protein that binds to the centromeric K-type repeat DNA of *Schizosaccharomyces pombe*. *Proc. Natl Acad. Sci. USA* **94,** 8427–8432 (1997).
6. Baum, M. & Clarke, L. Fission yeast homologs of human CENP-B have redundant functions affecting cell growth and chromosome segregation. *Mol. Cell. Biol.* **20,** 2852–2864 (2000).
7. Irelan, J. T., Gutkin, G. I. & Clarke, L. Functional redundancies, distinct localizations and interactions among three fission yeast homologs of centromere protein-B. *Genetics* **157,** 1191–1203 (2001).
8. Irvine, D. V. *et al.* Mapping epigenetic mutations in fission yeast using whole-genome next-generation sequencing. *Genome Res.* **19,** 1077–1083 (2009).
9. de Lahondes, R., Ribes, V. & Arcangioli, B. Fission yeast Sap1 protein is essential for chromosome stability. *Eukaryot. Cell* **2,** 910–921 (2003).
10. Mejia-Ramirez, E., Sanchez-Gorostiaga, A., Krimer, D. B., Schvartzman, J. B. & Hernandez, P. The mating type switch-activating protein Sap1 is required for replication fork arrest at the rRNA genes of fission yeast. *Mol. Cell. Biol.* **25,** 8755–8761 (2005).
11. Krings, G. & Bastia, D. Sap1p binds to Ter1 at the ribosomal DNA of *Schizosaccharomyces pombe* and causes polar replication fork arrest. *J. Biol. Chem.* **280,** 39135–39142 (2005).
12. Noguchi, C. & Noguchi, E. Sap1 promotes the association of the replication fork protection complex with chromatin and is involved in the replication checkpoint in *Schizosaccharomyces pombe*. *Genetics* **175,** 553–566 (2007).
13. Szilard, R. K. *et al.* Systematic identification of fragile sites via genome-wide location analysis of gamma-H2AX. *Nature Struct. Mol. Biol.* **17,** 299–305 (2010).
14. Aguilar-Arnal, L., Marsellach, F. X. & Azorin, F. The fission yeast homologue of CENP-B, Abp1, regulates directionality of mating-type switching. *EMBO J.* **27,** 1029–1038 (2008).
15. Arcangioli, B. & Klar, A. J. A novel switch-activating site (SAS1) and its cognate binding factor (SAP1) required for efficient mat1 switching in *Schizosaccharomyces pombe*. *EMBO J.* **10,** 3025–3032 (1991).
16. Deshpande, A. M. & Newlon, C. S. DNA replication fork pause sites dependent on transcription. *Science* **272,** 1030–1033 (1996).
17. Ghazvini, M., Ribes, V. & Arcangioli, B. The essential DNA-binding protein sap1 of *Schizosaccharomyces pombe* contains two independent oligomerization interfaces that dictate the relative orientation of the DNA-binding domain. *Mol. Cell. Biol.* **15,** 4939–4946 (1995).
18. Guo, Y. & Levin, H. L. High-throughput sequencing of retrotransposon integration provides a saturated profile of target activity in *Schizosaccharomyces pombe*. *Genome Res.* **20,** 239–248 (2010).
19. Segurado, M., Gomez, M. & Antequera, F. Increased recombination intermediates and homologous integration hot spots at DNA replication origins. *Mol. Cell* **10,** 907–916 (2002).
20. Meister, P. *et al.* Nuclear factories for signalling and repairing DNA double strand breaks in living fission yeast. *Nucleic Acids Res.* **31,** 5064–5073 (2003).
21. Sehgal, A., Lee, C. Y. & Espenshade, P. J. SREBP controls oxygen-dependent mobilization of retrotransposons in fission yeast. *PLoS Genet.* **3,** e131 (2007).
22. Kupiec, M. & Petes, T. D. Allelic and ectopic recombination between Ty elements in yeast. *Genetics* **119,** 549–559 (1988).
23. Gomez, E. B., Nugent, R. L., Laria, S. & Forsburg, S. L. *Schizosaccharomyces pombe* histone acetyltransferase Mst1 (KAT5) is an essential protein required for damage response and chromosome segregation. *Genetics* **179,** 757–771 (2008).
24. Huang, J. & Moazed, D. Association of the RENT complex with nontranscribed and coding regions of rDNA and a regional requirement for the replication fork block protein Fob1 in rDNA silencing. *Genes Dev.* **17,** 2162–2176 (2003).
25. Roguev, A. *et al.* Conservation and rewiring of functional modules revealed by an epistasis map in fission yeast. *Science* **322,** 405–410 (2008).
26. Ton-Hoang, B. *et al.* Single-stranded DNA transposition is coupled to host replication. *Cell* **142,** 398–408 (2010).
27. Wong, L. H. & Choo, K. H. Evolutionary dynamics of transposable elements at the centromere. *Trends Genet.* **20,** 611–616 (2004).
28. Putnam, C. D., Hayes, T. K. & Kolodner, R. D. Specific pathways prevent duplication-mediated genome rearrangements. *Nature* **460,** 984–989 (2009).
29. Wallis, J. W., Chrebet, G., Brodsky, G., Rolfe, M. & Rothstein, R. A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase. *Cell* **58,** 409–419 (1989).
30. Lemoine, F. J., Degtyareva, N. P., Lobachev, K. & Petes, T. D. Chromosomal translocations in yeast induced by low levels of DNA polymerase a model for chromosome fragile sites. *Cell* **120,** 587–598 (2005).

**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

**Acknowledgements** We thank the Martienssen laboratory, V. Aranda, E. Mejia-Ramirez and F. Antequera for technical advice and discussions, and R. Allshire, E. Noguchi, M. O'Connell, P. Espenshade and the National BioResource Project (T. Nakamura, Japan) for strains. This work was supported by National Institutes of Health grant RO1GM076396 to R.A.M., Cancer Research UK grant C9546/A6517 to J.B., l'Agence Nationale de la Recherche grant ANR-06-BLAN-0271 to B.A., a National Health and Medical Research Council C.J. Martin Fellowship to D.V.I. and a Postdoctoral Fellowship from the Spanish Ministry of Education to M.Z.

**Author Contributions** M.Z., B.A. and R.A.M. designed the experiments presented and wrote the paper. M.Z. performed and analysed the experiments. M.W.V. provided bioinformatic analysis. D.G. and D.V.I. provided strains. S.W. and J.B. performed additional experiments.

**Author Information** The sequences from the ChIP-seq experiments are deposited in Sequence Read Archive (www.ncbi.nln.nih.gov/sra) under accession number SRA024710.2. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to R.A.M. (martiens@cshl.edu).