Host genome surveillance for retrotransposons by transposon-derived proteins

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Transposable elements and their remnants constitute a substantial fraction of eukaryotic genomes. Host genomes have evolved defence mechanisms, including chromatin modifications and RNA interference, to regulate transposable elements. Here we describe a genome surveillance mechanism for retrotransposons by transposase-derived centromeric protein CENP-B homologues of the fission yeast *Schizosaccharomyces pombe*. CENP-B homologues of *S. pombe* localize at and recruit histone deacetylases to silence *Tf2* retrotransposons. CENP-Bs also repress solo long terminal repeats (LTRs) and LTR-associated genes. *Tf2* elements are clustered into 'Tf' bodies, the organization of which depends on CENP-Bs that display discrete nuclear structures. Furthermore, CENP-Bs prevent an 'extinct' *Tf1* retrotransposon from re-entering the host genome by blocking its recombination with extant *Tf2*, and silence and immobilize a *Tf1* integrant that becomes sequestered into Tf bodies. Our results reveal a probable ancient retrotransposon surveillance pathway important for host genome integrity, and highlight potential conflicts between DNA transposons and retrotransposons, major transposable elements believed to have greatly moulded the evolution of genomes.

Transposable elements, prevalent in most eukaryotic genomes, exert diverse effects on their hosts, profoundly influencing the organization, integrity and evolution of the host genome, and the host transcriptome^{1,2}. Host cells have devised strategies, such as DNA and histone methylation and RNA interference (RNAi), to control transposable element activity^{3–6}. Whereas RNAi has a prominent role in silencing transposable elements in most organisms, in mammals, an adaptive RNAi response, thus far, has been observed only in germ cells⁷. Moreover, RNAi in *S. pombe* is known to target preferentially a specific class of repeat (*dg* and *dh*) elements associated with constitutive heterochromatic regions, but RNAi and heterochromatin machineries have minor roles in silencing *Tf2* retrotransposons and solo LTRs⁸ dispersed throughout the genome^{9,10}. Therefore, additional RNAi-independent mechanisms probably exist to recognize and silence transposable elements.

The long evolutionary presence of transposable elements in eukaryotic genomes has resulted in many instances of hosts 'domesticating' transposable-element-encoded factors to perform cellular functions^{11–13}. Human CENP-B protein, which facilitates centromere formation by binding to short repeats within centromeric alpha satellite DNA^{14,15}, is derived from transposases of pogo DNA transposons^{16,17}. CENP-B, highly conserved in mammals, has homologues in other systems^{13,18}. The genome of *S. pombe* encodes three CENP-B homologues: Abp1, Cbh1 and Cbh2 (ref. 19). These have been shown to have redundant roles in centromeric heterochromatin formation and chromosome segregation^{19,20}. However, unlike cells lacking heterochromatin proteins such as Swi6/HP1, loss of Abp1 results in a slow-growth phenotype exacerbated by additional deletions of other CENP-B homologues^{18,19}, suggesting additional functions by CENP-Bs.

Here, we report roles for CENP-B homologues in the surveillance for retrotransposons and in genome organization in *S. pombe*. CENP-Bs bind to *Tf2* retrotransposons and their remnants, and mediate *Tf2* silencing by recruiting the class I histone deacetylase (HDAC) Clr6 (refs 21, 22) and the class II HDAC Clr3 (ref. 23).

CENP-Bs also repress several genes through nearby LTRs and facilitate HDAC recruitment to heterochromatic loci. We demonstrate that *Tf2* retrotransposons scattered across the genome are clustered into Tf bodies, the organization of which is mediated by CENP-Bs. This study highlights a potentially hitherto unrecognized host genome surveillance mechanism that exploits the targeting of a specific class of transposable elements by proteins of another class to rein in transposable element activities.

Genome-wide distributions of Abp1 and Cbh1

To gain insights into disparate phenotypes exhibited by mutations in the CENP-B family members of *S. pombe*, we mapped the distributions of these factors across the genome. Chromatin immunoprecipitation coupled with DNA microarray (ChIP–chip) analyses revealed Abp1 and Cbh1 binding at numerous loci on all three chromosomes (Fig. 1a). Consistent with the original finding of Abp1 association with certain ARS sites²⁴, we found Abp1 enrichment at *ars3002* (Supplementary Fig. 1a). Abp1 was also detected at centromeres (Supplementary Fig. 1b)^{19,20}. Close examination revealed preferential Abp1 enrichment at sequences immediately outside of *dh* repeats of centromere I and II, although low levels of binding were also observed at other sites (Supplementary Fig. 1b). Notably, Abp1 and Cbh1 were highly enriched at *Tf2* retrotransposons (Fig. 1a). Abp1 and Cbh1 co-localized throughout single, tandem and partial *Tf2* elements with both proteins displaying distinct binding peaks at the 5' and 3' LTRs (Fig. 1b and Supplementary Fig. 1c, d), suggesting a possible mode for recruiting these proteins to *Tf2* via specific Abp1/Cbh1-binding sites within *Tf2* LTRs. Conventional ChIP with primers positioned at unique sequences confirmed binding of Abp1 and Cbh1 at all 13 full-length *Tf2* elements and *Tf2* fragment 1 (Fig. 1b and Supplementary Figs 1c, d and 2). We also found that Abp1 and Cbh1 localized to solo LTRs, *wtf* elements (repeats often associated with *Tf* LTRs) and intergenic regions, many of which associate with the promoters of nearby genes (Fig. 1a, c and Supplementary Figs 3 and 4).

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CENP-Bs silence *Tf2* and LTR-associated genes

The binding of CENP-Bs to distinct genomic sites prompted us to investigate whether loss of these factors influences target loci expression. *Tf2* expression was monitored at two sites: LTR, which shares high sequence similarity with some solo *Tf2* LTRs, and coding region, which is present only in full-length *Tf2* and *Tf2* fragment 1 elements. Assays at both sites showed marked increases in *Tf2* expression in an *abp1Δ* strain (Fig. 2a). However, loss of *cbh1* or *cbh2*, or both, did not lead to detectable increases in *Tf2* expression; yet, single or double deletions of these genes in the *abp1Δ* background contributed to a slight further increase in *Tf2* expression relative to the *abp1Δ* mutant strain alone. We extended expression analyses to *wtf* elements and several genes close to solo LTRs enriched for CENP-Bs, and found that similar to *Tf2*, loss of Abp1 accounted for most of the increased levels of expression observed at *wtf* elements and genes associated with LTRs (Fig. 2b, c and Supplementary Fig. 4). These analyses suggest that among the *S. pombe* CENP-Bs, Abp1 is the member critically required for preventing widespread expression of *Tf2*, solo LTRs and LTR-associated genes, and thus could explain the marked growth defect observed in *abp1Δ* cells¹⁸.

Although both Abp1 and Cbh1 co-localize at *Tf2*, Abp1 alone is sufficient to repress *Tf2* expression, suggesting that Abp1 could be responsible for recruiting Cbh1 to *Tf2*. Indeed, the absence of either Cbh1 or Cbh2, or both, had no discernible effect on Abp1 binding to *Tf2* (Fig. 2d), whereas Cbh1, although unaffected by the absence of Cbh2, was delocalized from *Tf2* in cells lacking Abp1 (Fig. 2e). Immunoprecipitation assays showed Abp1 in complex(es) with Cbh1 and Cbh2 (Fig. 2f), supporting the idea that Abp1 recruits Cbh1, and possibly Cbh2, to *Tf2*.

Abp1 recruits HDACs to *Tf2* and a heterochromatic locus

The HDACs Clr3 (a component of the SHREC (Snf2/Hdac-containing repressor complex) silencing complex²³) and Clr6 (which

exists in multi-subunit complexes²¹) have been implicated in controlling *Tf2* expression in *S. pombe*^{10,21,23}. We investigated potential genetic interactions between HDACs and Abp1 in *Tf2* silencing. *Tf2* expression was increased and further elevated in *clr6* and double mutant *clr3 clr6* cells, respectively (Fig. 3a)¹⁰. However, cumulative increase in *Tf2* expression in double HDAC mutant cells was less compared to that observed in *abp1Δ* cells or in cells deficient for Abp1 and either Clr3 or Clr6 (Fig. 3a). These data indicate that Abp1 might act upstream by recruiting these HDACs to silence *Tf2*. Indeed, Abp1 enrichment at *Tf2* was not affected in single or double HDAC mutants, whereas Clr3 and Clr6 localizations at *Tf2* were compromised in *abp1Δ* cells (Fig. 3b, e). Also, Cbh1 localization at *Tf2* was partially affected in HDAC mutant cells (Fig. 3b), suggesting that HDACs might aid in the recruitment of Cbh1 to LTRs. We found Abp1 interacting with Clr3 and Clr6 *in vivo* (Fig. 3c, d). Together, these data show that Abp1 negatively regulates *Tf2* expression, in part, by directly recruiting HDACs to *Tf2*.

We next determined whether Abp1-mediated silencing of retrotransposons intersects with that of HIRA/Hip1 histone chaperone, implicated in *Tf2* silencing²⁵. The levels of *Tf2* expression were higher in *abp1Δ hip1Δ* cells compared with single mutant *abp1Δ* or *hip1Δ* cells (Fig. 3f), indicating that Abp1 and Hip1 might silence *Tf2* through distinct pathways. In this regard, Abp1 and Cbh1 localizations at *Tf2* were not affected in *hip1Δ* cells (Fig. 3g).

Heterochromatic silencing also involves HDAC recruitment to the target loci²⁶. Previous analysis revealed three major SHREC peaks at the mating-type (*mat*) region²³, two of which appear to correspond to silencer elements near the silent *mat* cassettes (Fig. 4a). The third peak overlaps with CENP-B binding outside of *cenH* (Fig. 4a), a known RNAi-dependent heterochromatin nucleation centre²⁶, suggesting that in addition to *Tf2*, CENP-Bs might also recruit SHREC at

Figure 1 | Genome-wide maps of Abp1 and Cbh1.

a, Chromosomal distribution profiles of Abp1 and Cbh1. Schematic diagrams of *S. pombe* chromosomes. ChIP assays were performed using strains expressing either Abp1-Flag(3×) or Cbh1-Flag(3×). ChIP-chip relative enrichments of Abp1 and Cbh1 were plotted against respective chromosomal position. Selective binding peaks of Abp1 and Cbh1 are indicated (IG, intergenic region; *tel*, telomere; *cen*, centromere; *mat*, mating-type locus). **b**, **c**, Abp1 and Cbh1 co-localize at *Tf2* and *wtf* elements. Abp1 and Cbh1 relative ChIP-chip enrichments at *Tf2* and *wtf* elements were confirmed with position-specific primers (black bars) by conventional ChIP PCR (right panels). WCE, whole cell extracts.

mat. Indeed, Clr3 binding was impaired in *abp1Δ* cells specifically at CENP-B binding sites (Fig. 4b). However, considerable levels of Clr3 binding remain across the silent *mat* region in *abp1Δ* cells, probably due to redundant recruitment mechanisms²⁶.

Dispersed *Tf2* elements cluster into Tf bodies

To gain further insights into the functions of CENP-Bs, we explored their subcellular localization. Immunostaining analyses showed that all three CENP-B proteins display complex nuclear 'network' structures (Fig. 5a and Supplementary Fig. 5a). In addition to localizing to 4,6-diamidino-2-phenylindole (DAPI)-stained nuclear regions, a dense concentration of CENP-Bs could be seen near the nucleolus. The congregation of CENP-Bs into nuclear networks prompted us to investigate whether *Tf2* elements are organized into higher-order structures. We tested this by performing fluorescence *in situ* hybridization (FISH) using probes directed against the coding region of *Tf2*. Notably, despite the presence of 13 full-length *Tf2* elements in the genome, only 1–3 *Tf2* foci could be seen per cell nucleus in wild-type cells (Fig. 5b), suggesting that CENP-Bs may have a role in clustering *Tf2* into specialized structures that we refer to as Tf bodies. Indeed, a substantial fraction of single and double mutant CENP-B cells contained more than three *Tf2* spots (Fig. 5b, c).

Mammalian CENP-Bs form homodimers through their conserved carboxy-terminal domain^{27,28}. We explored whether Abp1 could form dimers that might contribute to 'bundling' *Tf2* elements into Tf bodies. We found that Abp1 interacts with itself (Supplementary Fig. 6a), and this interaction depends on its dimerization domain. Unlike *abp1Δ*, which affects genomic stability that could potentially contribute to *Tf2* de-clustering, truncation of the Abp1 dimerization domain (*abp1-dm*) did not result in gross defects in growth or chromosome segregation (Supplementary Fig. 6b). Also, *abp1-dm* cells showed little or no change in *Tf2* expression (Supplementary Fig. 6c). However, *abp1-dm* cells were slightly defective in *Tf2* clustering, a phenotype that was further exacerbated when *cbh1* and *cbh2* deletions were combined with *abp1-dm* (Fig. 5d).

De-clustering of *Tf2* elements upon oxidative stress

Host cells subject to stress can activate proviruses and silent transposons^{29,30}. *Tf2* expression is known to be upregulated when cells are exposed to oxidative stress³¹. We assessed the effect of oxidative stress on the integrity of Tf bodies. Transient exposure of cells to hydrogen peroxide caused de-clustering of *Tf2* elements, despite CENP-Bs still being bound to *Tf2* (Supplementary Fig. 7a, b). It is possible that signalling pathways required for the cellular response to environmental stresses modify Tf bodies to facilitate rapid restructuring of the genome.

CENP-Bs target an extinct retrotransposon

In addition to *Tf2*, the *S. pombe* genome also contains 249 solo LTRs and LTR fragments, of which 11% belong to an extinct retrotransposon, *Tf1* (refs 8, 32). Because we found high levels of Abp1 and Cbh1 at *Tf1* LTRs, we hypothesized that CENP-Bs could also target *Tf1*. We tested this idea by reintroducing a full-length copy of *Tf1* into the genome. Previous work has shown that *Tf1* can insert into the genome either via integrase-mediated transposition or homologous recombination³³. Expression of *Tf1-neo* from a plasmid showed a minor increase in *Tf1* transposition in CENP-B mutant cells compared to wild-type cells (Supplementary Fig. 8a). However, we observed a marked increase in frequencies of genomic insertion by *Tf1* in cells lacking CENP-Bs, in particular *abp1Δ* or *cbh1Δ* mutants, when an integrase-defective *Tf1* was used (Supplementary Fig. 8a). This result indicated that Abp1 and Cbh1 could block the homologous recombination of *Tf1*. Sequencing of several *Tf1* integrants from *abp1Δ* and *cbh1Δ* cells showed *Tf1* recombination predominantly with existing *Tf2* elements or their remnants (Supplementary Fig. 8b). Therefore, in addition to silencing, CENP-Bs suppress recombination at *Tf* elements, which has important implications for maintenance of genomic integrity.

We next investigated whether the CENP-B-based surveillance mechanism can recognize a *Tf1* element integrated into the genome. Insertion of *Tf1* at *SPAC7D4.08*, a euchromatic gene, resulted in the targeting of CENP-Bs to this locus (Fig. 6a). CENP-Bs suppress

Figure 2 | CENP-Bs silence *Tf2*, *wtf* and several LTR-associated genes.

a, Increased *Tf2* expression in *abp1Δ* cells. Primers corresponding to LTRs flanking *Tf2* elements (*Tf2*-LTR) or *Tf2* coding region (*Tf2*-orf) were used for qPCR analysis (error bars indicate s.d.; $n = 4$; qPCR primers, black bar). **b**, **c**, Elevated expression of *wtf* (**b**) and *brl2* (**c**) in *abp1Δ* cells (error bars indicate s.d.; $n = 6$). **d**, **e**, Cbh1 localization at *Tf2* is dependent on Abp1 (**e**) but not vice versa (**d**). ChIP analyses of Abp1 and Cbh1 at a *Tf2* element in indicated mutant strains. ChIP fold enrichments relative to *act1* are shown below each lane. **f**, Abp1 interacts with Cbh1 and Cbh2. The input lanes are equivalent to 0.4% of the immunoprecipitation (IP) fraction. The anti-Flag input panel reflects longer film exposure than that of the immunoprecipitation.

transcription and transposition of the *Tf1* integrant, as indicated by increases in expression and transposition frequency of *Tf1* in the *abp1Δ* background (Fig. 6b, c). We also found that insertion of *Tf1* alters the localization of the *SPAC7D4.08* locus in the nucleus. Whereas *SPAC7D4.08* does not usually associate with Tf bodies, *Tf1* insertion at *SPAC7D4.08* resulted in a high incidence of *SPAC7D4.08* co-localizing with Tf bodies (Fig. 6d and Supplementary Fig. 5b), probably as a result of *Tf1* being 'bundled' into Tf bodies.

Discussion

Our study uncovers unexpected targeting of *S. pombe* CENP-B homologues to *Tf* retrotransposons and their remnants to mediate both transcriptional and recombinational repression. CENP-Bs thought to have originated from transposases encoded by an ancient pogo-like DNA transposon^{16,17} could hamper the mobility of LTR retrotransposons (this study). Given that both DNA transposons and LTR retrotransposons are flanked by repetitive DNA structures, and transposases bind to terminal inverted repeats of DNA transposons², it is possible that during evolution a CENP-B precursor acquired the ability to target retrotransposon LTRs, and subsequently was co-opted by the host into its gene repertoire for controlling transposable elements.

CENP-B-mediated *Tf* silencing is, in part, dependent on CENP-Bs recruiting Clr3-containing SHREC and Clr6 HDAC complexes, which are also required for heterochromatic silencing of centromeric

repeats^{10,21,23}. Thus, CENP-B localization at heterochromatic regions could aid in silencing by means of HDAC recruitment. Indeed, Abp1 mediates SHREC recruitment to the silent *mat* region. The targeting of HDACs via CENP-B might facilitate assembly of 'closed' chromatin that not only represses transcription but also protects genomic integrity by rendering repetitive sequences recombinationally inert^{23,26,34}.

Considering that heterochromatin and CENP-Bs recruit a common set of silencing factors, CENP-B regulation of *Tf* elements might represent a 'simple' form of local heterochromatic silencing. In this respect, it would not be surprising that *Tf2* silencing might use other heterochromatin and RNAi components whose normally minor or redundant roles manifest under specific conditions. *S. pombe* CENP-Bs may recruit distinct effectors. Apart from the recruitment of HDACs to *Tf* elements, CENP-Bs might target additional factors critical for transposable element surveillance. As Abp1 and Cbh1 can bind to distinct loci, and that these factors do not always co-localize with SHREC²³, CENP-B binding in contexts other than heterochromatin and transposable elements might recruit activities important for other chromatin transactions, such as transcription and DNA replication.

Figure 3 | Abp1 directly recruits HDACs Clr3 and Clr6 and coordinates with Hip1 to silence *Tf2*. **a, f**, qPCR analyses of *Tf2* expression in cells carrying single or combinational mutations of *abp1* with HDACs (**a**), or *hip1* (**f**) (error bars indicate s.d.; $n = 6$). **b, g**, Effects of mutations of HDACs (**b**) and *hip1* (**g**) on Abp1 and Cbh1 localizations at *Tf2-11* were analysed by ChIP assays. **c, d**, Clr3 and Clr6 interact with Abp1. The input lanes are equivalent to 0.1% (**c**) and 0.2% (**d**) of immunoprecipitation fractions. Anti-Flag input panels reflect longer film exposure than that of the immunoprecipitation. **e**, Clr3 and Clr6 localizations at *Tf2-12* in wild-type and *abp1Δ* cells were analysed by ChIP assays. Control corresponds to a gene (*SPBC1348.13*) containing little enrichment for HDACs and CENP-B proteins.

Figure 4 | CENP-Bs recruit the SHREC subunit Clr3 to the silent mating-type locus. **a**, ChIP-chip results of CENP-Bs at *mat* were overlaid with those of SHREC subunits Clr2 and Clr3 (ref. 23), showing their co-localization just outside of *cenH*, an RNAi-mediated heterochromatin nucleation site²⁶. Two other SHREC-binding peaks overlap with *mat* silencer elements REII and REIII; the latter contains a binding site for Atf1/Pcr1, involved in Clr3 localization. IR-L and IR-R denote left and right inverted repeat boundary elements flanking the silent *mat* interval, respectively²⁶. **b**, Clr3 recruitment at *mat* was impaired in *abp1Δ* cells. Clr3 *mat* distribution in *abp1Δ* cells determined by ChIP-chip was overlaid with that in wild-type cells. Reduced Clr3 binding at *mat* in *abp1Δ* cells and localization of CENP-Bs at *mat* were confirmed by conventional ChIP assays (right panels) with position-specific primers (black bars). Control corresponds to a gene (*SPBC1348.13*) containing little enrichment for HDACs and CENP-B proteins.

Figure 5 | Discrete CENP-B nuclear distributions and formation of Tf bodies. **a**, Immunofluorescent analyses of Myc-tagged CENP-Bs. **b**, **c**, *Tf2* elements cluster into Tf bodies, and *Tf2* de-clustering is evident in CENP-B mutants. Shown are merged FISH images of *Tf2* and DAPI of two representative cells from each strain. Quantitative FISH analyses (**c**) were performed on the indicated number of cells (*n*) for respective strains. **d**, The Abp1 dimerization domain facilitates *Tf2* clustering. Quantitative FISH analyses of *Tf2* in indicated strains.

Accumulating evidence implicates transposable-element-derived sequences as regulators of gene expression in diverse species^{1,35–37}. A substantial fraction of human promoters contains transposable element sequences³⁸, and instances of their contribution to gene regulation have been documented^{1,39}. We provide evidence that CENP-Bs bound to LTRs can regulate expression of nearby genes, presumably owing to their ability to recruit chromatin modifiers. CENP-Bs also occupy several gene promoters, that, at one time, might have contained transposable element sequences, but most of which have decayed beyond recognition. Therefore, in addition to retrotransposon surveillance, CENP-Bs and their targeted transposable element sequences might serve as versatile regulatory modules, enhancing the ability of cells to modulate gene regulatory networks³⁵.

Our analyses revealed unexpected clustering of *Tf2* elements into Tf bodies. These observations are reminiscent of *Drosophila* gypsy retrotransposons brought together into specialized bodies to facilitate chromatin organization⁴⁰. Moreover, transposon-derived MAR/SAR sequences³⁸ have been shown to create chromatin loops⁴¹. These findings indicate a conserved role for transposable elements in genome organization. Dimerization of CENP-B proteins⁴² (this study) bound to LTRs may directly or indirectly promote either local or long-range loops between LTRs of individual *Tfs* or different *Tf* elements. As such, enrichment of CENP-B proteins at *Tf2* peaks at flanking LTRs but progressively decreases towards the centre of *Tf2* elements. The clustering of *Tfs* into distinct bodies may facilitate transposable element surveillance or other genome-wide processes including prevention of aberrant recombination, coordinated transcriptional control and genome reorganization in response to environmental stresses (Fig. 4g)²⁹.

This study may have implications for several phenomena observed in other systems. *Drosophila* P and 1360 elements can trigger heterochromatic silencing that is highly dependent on element copy number^{43,44}. This mass action requirement for silencing might reflect a

Figure 6 | Genome surveillance controls by CENP-Bs against an introduced extinct *Tf1* element. **a**, Targeting of CENP-Bs to a genomic site containing a *Tf1* integrant. ChIP assays of Abp1 and Cbh1 in strains with or without a *Tf1* integrant at *SPAC7D4.08* is shown. **b**, **c**, Increases in expression (error bars indicate s.d.; *n* = 3) (**b**) and transposition frequency (**c**) of *Tf1* integrant in *abp1Δ* cells. qPCR (**b**) and transposition (**c**) assays (see Methods) of *Tf1* in the indicated mutant strains is shown. **d**, Association of *Tf1* with Tf bodies. Co-FISH analyses with *Tf2*-cy3 (red) and *SPAC7D4.08*-Alexa-488 (green) probes in strains with and without the *Tf1* integrant is shown. **e**, Model of *S. pombe* CENP-Bs in surveillance controls of LTR retrotransposons. CENP-B binding to *Tf* LTRs leads to recruitment of HDACs and presumably other silencing activities that contribute to *Tf* silencing, whereas CENP-B dimerization directly or indirectly promotes *Tf2* clustering.

high concentration of specific transposable-element-binding proteins similar to CENP-Bs capable of recruiting chromatin modifiers. The formation of Tf bodies could have parallels in mammalian X-chromosome inactivation, in which transposable elements might facilitate the assembly of a silent nuclear compartment^{45–47}. Recent evidence suggests that whereas RNAi has a prominent role in silencing transposable elements in germ cells in higher eukaryotes⁴⁸, there are probably alternative mechanisms, possibly similar to that of *S. pombe* CENP-B-based surveillance, for regulating transposable elements in somatic cells.

METHODS SUMMARY

Strains were generated by standard yeast methods. ChIP and ChIP–chip were performed as previously described⁹. Quantitative PCR (qPCR) expression analysis was done using a two-step real-time RT–PCR. Mobility of a *Tf1* integrant was assessed using *Tf1-neo* strains carrying an artificial intron (*AI*)^{48,49} inserted in the opposite orientation of *neo*, thereby inactivating *neo*. Retrotransposition rate was determined by fluctuation analysis⁵⁰ of cells regaining *neo* owing to *AI* loss during *Tf1* retrotransposition. Sequences of primers used are available in Supplementary Table 1.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Information Microarray data are available at NCBI GEO repository under the accession number GSE9056 and at NCI (<http://pombe.nci.nih.gov/genome/>). Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.I.S.G. (grewals@mail.nih.gov)

METHODS

S. pombe strains. *abp1Δ*, *hip1Δ* and C-terminal-tagged strains (*Abp1*–Flag(3×), *Cbh1*–Flag(3×), *Abp1*–Myc(13×), *Cbh1*–Myc(13×), *Cbh2*–Myc(13×)) were constructed according to a PCR-based method. The *Tf1-neoAI* strain was generated by transforming a strain containing *Tf1-neo* integrated at *SPAC7D4.08* with a PCR *neo* fragment containing an artificial intron (*AI*)^{48,49} positioned in reverse orientation to *neo*; neo-sensitive colonies were selected, and *AI* insertion into *neo* was confirmed by DNA sequencing. *abp1Δ::leu2*, *cbh1Δ::leu2* and *cbh2Δ::leu2* strains were gifts from Y. Murakami. All other strains were obtained by standard genetic crosses.

ChIPs. ChIP and ChIP–chip experiments were performed as previously described⁹ with minor modifications. Sonicated cross-linked chromatin (50 ml *S. pombe* culture (optical density (OD) 0.5–1) cross-linked with 3% paraformaldehyde for 30 min at 18 °C followed by 10 mM dimethyl adipimidate for 45 min at room temperature) was immunoprecipitated with anti-Myc (9E10, BD Biosciences; A-14, Santa Cruz Biotech) antibody for *Clr3*–Myc, or anti-Flag-coupled beads (M2, Sigma-Aldrich) for *Abp1*–Flag and *Cbh1*–Flag. Antibody used against *Clr6* has been described previously²¹. For conventional ChIP assay, ChIP PCR products (26–30 cycles) were analysed by gel electrophoresis (4% polyacrylamide gel) and visualized with SYBR Green (Invitrogen) staining. Relative ChIP enrichment was quantified using a Typhoon scanner and ImageQuant software.

Quantitative real-time PCR (qPCR). A total of 500 ng of DNase-treated total RNA isolated by MasterPure yeast RNA purification kit (Epicentre) was reverse transcribed (RT) with Superscript III and oligo(dT)₂₀ primer (Invitrogen). Complementary DNA (1/50 RT reaction) was used as templates for SYBR green (0.1× dilution) qPCR analysis (Opticon 2). Relative fold expressions relative to *act1* were determined in Excel.

Immunoprecipitation. A total of 50 ml *S. pombe* culture (OD 1–1.5) was lysed in HCS buffer (150 mM HEPES pH 7.2, 250 mM NaCl, 0.1% NP-40, 20 mM (NaF, BGP), 1 mM (EDTA, dithiothreitol, PMSF) and protein inhibitor tablet (Roche)) by bead beater (three times 30 s with 2 min interval on ice). Protein extracts (1–5 mg) from indicated strains pre-cleared in protein A/G beads were incubated for 2 h at 4 °C with M2 agarose beads (Sigma-Aldrich), extensively washed afterwards with HCS buffer and subjected to PAGE gel (NUPAGE Novex 10% BT, Invitrogen) and western blot analyses (iBlot, Invitrogen) with either anti-Myc (NB600-336, Novus Biologicals), anti-Flag (M2, Stratagene) or anti-*Clr6* antibodies.

Imaging analysis. For immunofluorescent analysis, 10 ml *S. pombe* cells (OD 0.5–1) were cross-linked in 1.2 M sorbitol with 3.8% paraformaldehyde at 18 °C for 30 min, quenched with 125 mM glycine, and subjected to cell-wall digestion with zymolase (Seikagaku), and blocked with PEMBAL (100 mM PIPES pH 6.9, 1 mM EGTA, 1 mM MgSO₄, 1% BSA, 0.1 M L-lysine) for 1 h before incubation with antibody. Anti-Myc (9E10, BD Biosciences) antibody and Alexa-Fluor-488-conjugated goat anti-mouse antibody (Invitrogen) were used to detect Myc-tagged CENP-B. For FISH analysis, cells were treated similarly as those for immunofluorescent analysis. After blocking with PEMBAL, cells were treated with RNase A (0.1 μg μl^{−1}) at 37 °C for at least 3 h. Hybridization was carried out with 100–150 ng of probes in 100 μl hybridization buffer (50% formamide, 2× SSC, 5× Denhart's solution, 10% dextran sulphate) at 40 °C for 10–12 h. Cells were washed three times in 100 μl 2× SSC for 30 min each before mounting. For FISH probe preparation, a 3.0-kb or 4.3-kb PCR fragment corresponding to the coding region of a full-length *Tf2* element or the upstream promoter region of *SPAC7D4.08*, respectively, was generated by PCR. *Tf2* PCR fragment was subsequently cloned into a TOPO II vector (TOPO II *Tf2*-orf). *Tf2*-orf plasmid DNA or purified *SPAC7D4.08* PCR product was digested with *AluI* and *DdeI* restriction enzymes and labelled by random priming (Takara 6045) with either dCTP-cy3 (*Tf2*) or amino-allyl dUTP/Alexa 488 (*SPAC7D4.08*). Statistical significance of *Tf2* de-clustering in *abp1* mutant cells relative to those of wild-type ($P < 0.05$) was assessed using a one-way analysis of variance test. For image deconvolution, immunofluorescent analysis and FISH images were collected at 0.2 μm intervals along the Z-axis and subjected to volume deconvolution using the nearest neighbour method (Improvision, OPENLAB).

Transposition assay. Wild-type and CENP-B mutants were assayed for *Tf1* integration as described previously⁴⁹. For mobility assay of a *Tf1* integrant, 2×10^5 cells of wild-type or *CENP-BA* strains carrying *SPAC7D4.08::Tf1-neoAI* were inoculated in ten parallel 2-ml cultures and grown at 30 °C for 2–3 days. 1×10^8 cells from each culture were plated on a G418 plate (150 μg ml^{−1}), and neo-resistant colonies were scored. Cells from five cultures were titred on YEA plates. Transposition rate was determined by fluctuation analysis⁵⁰.