Restriction of Retrotransposon Mobilization in <i>Schizosaccharomyces pombe</i> by Transcriptional Silencing and Higher-Order Chromatin Organization

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ABSTRACT

Uncontrolled propagation of retrotransposons is potentially detrimental to host genome integrity. Therefore, cells have evolved surveillance mechanisms to restrict the mobility of these elements. In <i>Schizosaccharomyces pombe</i> the T2 LTR retrotransposons are transcriptionally silenced and are also clustered in the nucleus into structures termed Tf bodies. Here we describe the impact of silencing and clustering on the mobility of an endogenous T2 element. Deletion of genes such as set1+ (histone H3 lysine 4 methyl-transferase) or abp1+ (CENP-B homolog) that both alleviate silencing and clustering, result in a corresponding increase in mobilization. Furthermore, expression of constitutively active Sre1, a transcriptional activator of T2 elements, also alleviates clustering and induces mobilization. In contrast, clustering is not disrupted by loss of the HIRA histone chaperone, despite high levels of expression, and in this background, mobilization frequency is only marginally increased. Thus, mutations that compromise transcriptional silencing but not Tf bodies are insufficient to drive mobilization. Furthermore, analyses of mutant alleles that separate the transcriptional repression and clustering functions of Set1 are consistent with control of T2 propagation via a combination of silencing and spatial organization. Our results indicate that host surveillance mechanisms operate at multiple levels to restrict T2 retrotransposon mobilization.

KEYWORDS

<i>Schizosaccharomyces pombe</i>; T2 LTR retrotransposons; higher-order chromatin organization; retrotransposition; transcriptional silencing

LTR retrotransposons are virtually ubiquitous in eukaryotes and have had major impacts upon host genome evolution, organization, and function (Kazazian 2004). They are structurally related to exogenous and endogenous retroviruses and are composed of LTR sequences that flank genes encoding, Gag, protease (PR), reverse transcriptase (RT) and integrase (IN) proteins (Beauregard et al. 2008). Retrotransposon RNA is synthesized by host RNA polymerase II from a promoter in the 5′ LTR. The resulting messenger RNA (mRNA) serves as a template for the translation of retrotransposon proteins and also for reverse transcription. Reverse transcription occurs within a virus-like particle and the resulting complementary DNA (cDNA) is inserted into the genome by the element-encoded IN or by homologous recombination (Beauregard et al. 2008). The insertion of retrotransposon cDNA is inherently mutagenic with potentially deleterious effects on the host (Levin and Moran 2011; Burns and Boeke 2012). Furthermore, the repetitive nature of retrotransposons renders them substrates for recombination and potential drivers of genome rearrangements. As a result, these elements have traditionally been viewed as harmful genomic parasites (Orgel and Crick 1980). However, there are numerous examples where host cells have domesticated transposon proteins or sequences for their own use, a process termed exaptation (Shapiro 2005; Chalopin et al. 2015).
Epigenetic controls that suppress the transcription of retroelements, play a key role in preventing their uncontrolled spread (Maksakova et al. 2008). DNA methylation, RNA interference (RNAi), histone modification, and chromatin remodelling have all been implicated in the suppression of specific families of LTR retrotransposons and endogenous retroviruses (Slotkin and Martienssen 2007; Maksakova et al. 2008; Levin and Moran 2011). However, in many cases, the controls that regulate expression of these elements are incompletely understood. Moreover, genetic studies indicate that the mobilization of LTR retrotransposons is subjected to multilayered regulation (Maxwell and Curcio 2007).

Analyses of yeast species such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have provided fundamental insights into LTR retrotransposon biology (Kelly and Levin 2005; Lesage and Todeschini 2005). The genome of the common laboratory strain of *S. pombe* (972) contains a highly homogenous group of 13 Tf2 LTR retrotransposons, which belong to the Ty3/Gypsy family (Bowen et al. 2003; Essnault and Levin 2015). A closely related element called Tf1 is present in other wild strains but full-length copies of this element are absent in the laboratory strain 972 (Bowen et al. 2003; Essnault and Levin 2015). However, there is an extensive population of ~250 solo LTRs in this strain, which includes sequences derived from other Tf families, including Tf1. Sequence analysis indicates that the majority of the Tf2 elements have the potential to be active and Tf2-12 has been shown to mobilize with a frequency of approximately two new insertions per 10^8 cells (Sehgal et al. 2007). This low rate of mobilization is consistent with low levels of Tf2 transcription in wild-type (WT) cells grown under standard conditions. Indeed, a number of studies indicate that the expression of Tf2 retrotransposons is subjected to chromatin-mediated silencing by a variety of factors including CENP-B proteins (Cam et al. 2008), the Set1 histone methyltransferase (Lorenz et al. 2012), multiple histone deacetylases (Hansen et al. 2005; Durand-Dubief et al. 2007; Nicolas et al. 2007; Cam et al. 2008), and the histone chaperones HIRA and Asf1 (Greenall et al. 2006; Anderson et al. 2009; Yamane et al. 2011). Interestingly, the RNAi machinery plays only an accessory role to the exosome in this process (Cam et al. 2005; Hansen et al. 2005; Yamanaka et al. 2013).

The role of the CENP-B homologs represents an interesting instance of competition, as these proteins are derived from a transposase derived from an ancient DNA transposon (Irelan et al. 2001). In addition to their roles in Tf2 silencing, CENP-B and Set1 also function to cluster Tf2 elements and solo LTRs into subnuclear structures called Tf bodies (Cam et al. 2008; Lorenz et al. 2012; Mikheyeva et al. 2014). These bodies are not apparently necessary for silencing of Tf2 elements, but they have been proposed to prevent integration via recombination of other Tf elements (Cam et al. 2008; Mikheyeva et al. 2014).

In order to further investigate the host cell controls that restrict Tf2 LTR retrotransposons, we have constructed a sensitive reporter assay that enables us to monitor the mobilization of an endogenous Tf2 element. Rather than relying on the plasmid-encoded elements expressed from heterologous promoters that could circumvent the transcriptional controls to which endogenous native elements are subjected, this assay enabled us to determine the impact of mutations in key regulatory genes upon the mobilization frequency of an endogenous Tf2 element. We find that mutations that compromise both transcriptional repression and also Tf body formation result in elevated mobilization rates. However, loss of silencing in the presence of intact Tf bodies is not sufficient to render high levels of mobilization. Our results, therefore, highlight that the mobility of LTR retrotransposons are subjected to regulation at multiple levels and suggest that higher-order chromatin organization is an important aspect of host cell control.

**Materials and Methods**

**Strains**

The natAI cassette was constructed by inserting a double-stranded oligonucleotide corresponding to the 37-bp intron of the nda3+ gene into the NruI site of pFA6-natMX6 (Wach 1996) to give pFA6-natAI. Tagging of the Tf2-12 element with natAI was achieved by assembling the following DNA fragments in pGEM-T: 0.43 kb of Tf2-12 (3576–4006 bp), 1.2 kb natAI cassette and a 0.39-kb sequence containing the 3’ UTR and LTR of Tf2-12 (4010–4400 bp) and 0.47 kb of chromosomal sequence downstream of Tf2-12. The resulting DNA fragment was released from the pGEM-T vector and used to transform strain AS50 (Sehgal et al. 2007). Colonies resistant to 5-FOA were isolated and correct integration of the fragment at the Tf2-12 locus was confirmed by PCR. Two independent Tf2-12natAI strains were retained and used as the parental strains for mobilization assays. Mutations were introduced into the Tf2-12natAI background by standard genetic crosses. Strains used in this study are described in Supplemental Material, Table S1.

**Tf2-12natAI mobilization assays**

Strains were plated onto YESS (yeast extract 5 g/L, glucose 30 g/L, histidine, adenine, uracil, leucine and lysine hydrochloride 225 mg/L) agar to give well-dispersed single colonies. A small (≤1 mm) colony was used to inoculate a 12-ml YESS culture, which was then incubated at 30° with shaking until the culture had reached saturation (~48 hr). A 10-ml aliquot was harvested, resuspended in 500 μl H2O, and plated onto two YESS agar plates supplemented with nourseothricin (Nat) (75 μg/ml). An aliquot of the remaining culture was then subjected to 10-fold serial dilution and aliquots of the 10^-5, 10^-6, and 10^-7 dilutions were plated onto YESS agar plates. Plates were incubated at 30° for 3–4 days to allow colonies to form. The proportion of Nat-resistant cells as a fraction of the total viable cells was used to calculate Tf2-12natAI mobilization frequency. For each strain under analysis, the mobilization frequency of five independent cultures was measured and the median value determined. This process was repeated a minimum of three times for each strain under analysis and a mean mobilization frequency was calculated from the median values.
For the WT background, the mean mobilization frequency is derived from 13 median values. P-values were generated by pairwise comparisons using a t-test.

**β-Galactosidase assays**

Strains harboring a Tf2-lacZ reporter (Anderson et al. 2009) were grown in rich (YE5S) medium at 30°C until they reached an OD595 of between 0.3 and 0.5. Cells were then harvested and processed for β-galactosidase assays as described previously (Guarente 1983). For each strain under study, the mean β-galactosidase activity was determined from at least three independent biological repeats each one assayed in duplicate. Values were scaled relative to the WT control.

**FISH analysis**

FISH assays were performed as previously described (Mikheyeva et al. 2014). Briefly, 10 ml of cells (OD595 ~0.5–1; YEA media [yeast extract 5 g/L, glucose 30 g/L, adenine 75 mg/L, uracil 225 mg/L, leucine 225 mg/L, histidine 225 mg/L and lysine hydrochloride 225 mg/L]) diluted with 10 ml of 2.4 M sorbitol YEA were cross-linked with 2.9 ml of freshly made 30% paraformaldehyde/YEA solution for 30 min in a 18°C water bath shaker and subsequently quenched with 1.2 ml of 2 M glycine. Cells were subjected to cell wall digestion (0.5 mg/ml zymolyase solution for 1 hr) followed by RNase A treatment (0.1 mg/ml at 37°C for 3 hr). Cells were hybridized with 100–150 ng dCTP-Cy3-labeled Tf2-ORF probes in 100 μl hybridization buffer (50% formamide, 2× SSC, 5× Denhart's solution, 10% dextran sulfate) at 40°C for 12–14 hr followed by washing three times with 100 μl 2× SSC for 30 min at room temperature. Nuclei were visualized by DAPI staining in 1× PBS for 5 min at room temperature. Images were obtained using a Zeiss Axioplan 2 microscope. The χ²-test of homogeneity was used to determine whether declustering of Tf2 elements seen in mutant cells relative to WT was significant.

**RNA analysis**

RNA was extracted using hot phenol and purified over RNase easy columns (QIAGEN, Valencia, CA) as previously described (Lyne et al. 2003). For strand-specific RT-PCR, one primer complementary to the sense or antisense transcript was added during first strand cDNA synthesis, while the second primer was added prior to the PCR amplification steps. cDNA for quantitative PCR (qPCR) was made using a Superscript II kit (Invitrogen, Carlsbad, CA). qPCR reactions were performed using a LightCycler 2.0 PCR system (Roche Diagnostics, Indianapolis, IN) and SYBR Green mix (Molecular Probes, Eugene, OR) using the appropriate primers.

**Southern blotting**

Southern blotting of NcoI- and HindIII-digested genomic DNA was performed as previously described (Prudden et al. 2003). A DNA probe specific for natAI was amplified by PCR using pFA6a-natAI as a template and oligonucleotide primers 5′-CAGAGAACAAGTACCTAC-3′ and 5′-TCGCCCTGACATCATACTGC-3′.

**Western blotting**

Western blotting was carried out as previously described (Mikheyeva et al. 2014). Briefly, 50 μg of protein extract was used for SDS/PAGE analysis followed by Western blotting. Tf2
of at least three independent assays and are scaled relative to the WT value. Error bars indicate ± SEM. (C) The mobilization frequency of Tf2-12natAI was determined by fluctuation analysis as described in Materials and Methods. Values were scaled relative to the WT. Error bars indicate ± SEM. *** P < 0.001 (t-test).

IN was detected using a rabbit antiserum against Tf1 IN at a dilution of 1:1000.

**Data and reagent availability**

All strains are available upon request.

**Results**

In order to analyze the host cell mechanisms that control the propagation of endogenous Tf2 LTR retrotransposons, we tagged the Tf2-12 element with an antibiotic nat-resistance cassette, which is disrupted with an artificial intron (natAI). The intron is orientated in the same transcriptional direction as that of Tf2-12 but opposite to that of the nat cassette, therefore a functional nat cassette is generated only after successful intron splicing from the Tf2-12natAI transcript and integration of the processed Tf2 element (Figure 1A). Cells that undergo a mobilization event become resistant to Nat allowing mobilization frequency to be determined from the proportion of Nat-resistant colonies in the population. Southern blotting confirmed that the acquisition of Nat resistance was accompanied by a genuine mobilization event (Figure 1B). We found that this element mobilizes with low frequency (2.06 × 10^{-8}/cell) in WT cells grown under normal conditions, consistent with a previous study for an endogenous Tf2 (Sehgal et al. 2007). Mobilization was also severely reduced in cells lacking the RecA homolog, Rad51(Rhp51) (Figure 1C), confirming Tf2’s preferred mode of genome insertion via homologous recombination rather than by IN-mediated integration (Hoff et al. 1998; Sehgal et al. 2007).

**Constitutive transcriptional activation drives Tf2 mobilization**

LTR retrotransposons and endogenous retroviruses are commonly quiescent under normal growth conditions but are differentially activated in response to environmental stress stimuli (Lesage and Todeschini 2005; Cho et al. 2008; Grandbastien 2014). Indeed, previous studies have revealed that Tf2 elements are activated by a low oxygen environment (Sehgal et al. 2007) and we showed that Tf2-12natAI was activated under hypoxic conditions (Figure 2A). The response of Tf2 elements to oxygen is dependent upon the transcription factor Sre1, which is an ortholog of mammalian sterol element binding protein (SREBP). Under normal oxygen conditions, Sre1 is bound to membrane in the ER but low oxygen levels result in the proteolytic cleavage of the N-terminal domain, which translocates to the nucleus and activates transcription via SRE elements in Tf2 LTRs and other oxygen-responsive promoters (Sehgal et al. 2007; Hughes and Espenshade 2008). In order to determine whether Sre1-mediated activation is sufficient to induce mobilization, or whether additional facets of hypoxic conditions are required, we employed a strain (sre1-N) that expresses a constitutively active form of Sre1 (Hughes and Espenshade 2008). As expected, the sre1-N allele induced the expression of an integrated Tf2-lacZ reporter (13-fold) (Figure 2B). Furthermore, this was closely correlated with a 20-fold increase in Tf2 mobilization (Figure 2C). Thus, the controls that restrict retrotransposon mobilization during normal growth conditions can be circumvented by active Sre1.

**Set1 methyltransferase and CENP-B homolog Abp1 restrict Tf2 mobilization**

Eukaryotic cells often immobilize retroelements in repressive chromatin structures, and this is believed to be a key mechanism that suppresses their expression and thereby restricts their spread (Slotkin and Martienssen 2007; Maksakova et al. 2008). Although the expression of Tf2 elements is repressed under normal growth conditions, these elements are found exclusively within euchromatin, which likely reflects the preference of Tf elements to integrate near Pol II promoters. We have previously shown that Tf2s are enriched with histone H3 lysine 4 methylation (H3K4me) (Noma and Grewal 2002; Cam et al. 2005). This euchromatin mark is mediated by Set1 and cells deficient in set1+ fail to repress Tf2s (Lorenz et al. 2012). We therefore assessed the role of Set1 in the control of Tf2 mobilization. Consistent with previous reports, deletion of set1+ resulted in a significant (eightfold) increase in Tf2-lacZ expression and also a significant (fivefold) increase in the frequency of Tf2-natAI. **Figure 2** Constitutive activation of the SREBP homolog Sre1 results in high levels of Tf2 mobilization. (A) Cells were patched onto YESS plates and incubated at 30° for 2 days either under normal oxygen conditions or in an anaerobic jar. Cells were then resuspended in H2O and ~1 × 10^8 cells spread onto YESS plates supplemented with Nat. Plates were incubated at 30° under normal oxygen conditions until colonies appeared. (B) The indicated strains were grown to midlog growth phase at 30° in YESS. Cells were harvested and processed for β-galactosidase assays. Results are the mean...
mobilization (Figure 3, A and B). We conclude that Set1 functions to both restrict the expression and the mobilization of Tf2 elements.

Transcriptional silencing of Tf2 elements is also dependent upon CENP-B homologs (Abp1, Cbh1, and Cbh2), which localize to Tf2 LTRs and mediate the recruitment of class I and II HDACs (Cam et al. 2008; Zaratiegui et al. 2011; Lorenz et al. 2012). CENP-Bs have been shown to restrict the genomic reintegration of an “extinct” retrotransposon, Tf1 (Cam et al. 2008); therefore, we investigated their roles in regulating the spread of an endogenous Tf2 element. We determined the impact of deleting abp1Δ, the most prominent CENP-B member, and as previously reported, found increased expression of Tf2 (Figure 3, C and D). Moreover the increased level of expression in the abp1Δ background was accompanied by a similar (~4-fold) increase in the frequency of Tf2-12natAI mobilization (Figure 3B). As CENP-Bs recruit HDACs to Tf2 LTRs, we next determined the frequency of Tf2-12natAI mobilization in a clr6-1 background, which harbors a point mutation in an essential class I HDAC (Grewal et al. 1998). Previous studies have shown that the clr6-1 allele is associated with a moderate derepression of Tf2 elements (Hansen et al. 2005) and consistent with this finding, the frequency of Tf2-12natAI was modestly increased (2.5-fold) in this background (Figure 3B). Under standard growth conditions, the RNAi machinery plays only a minor role in restricting Tf2 expression (Cam et al. 2005; Hansen et al. 2005; Yamanaka et al. 2013). Consistent with this, loss of the RNA processing enzyme Dcr1 did not result in increased Tf2 mobilization (Figure 3B). Taken together, these results indicate that the propagation of Tf2 LTR retrotransposons is restricted by the combined functions of Set1, CENP-Bs, and HDACs.

Loss of HIRA-mediated transcriptional silencing does not result in increased Tf2 mobilization

It has been demonstrated that the expression of all 13 Tf2 elements is repressed by the HIRA histone chaperone complex and that loss of any one of the four HIRA complex subunits (Hip1, Slm9, Hip3, or Hip4) results in a dramatic increase in Tf2 RNA (Greenall et al. 2006; Anderson et al. 2009; Anderson et al. 2010; Yamane et al. 2011). We therefore compared Tf2 expression and mobilization in a background that lacks HIRA function (hip1Δ). Surprisingly, despite a very large increase in Tf2-lacZ expression (41-fold) we observed only a very modest (1.7-fold) increase in the frequency of Tf2 mobilization in hip1Δ cells (Figure 4, A and B). Furthermore, deletion of the genes encoding the other HIRA complex subunits (slm9Δ, hip3Δ, and hip4Δ) did not result in increased Tf2-12natAI mobilization relative to WT cells. Indeed, mobilization was decreased in hip3Δ cells (Figure 4C). In order to rule out the possibility that the expression of Tf2-12natAI is not properly regulated by HIRA, we used strand-specific RT-PCR to measure Tf2-12natAI transcript levels and found that they were markedly increased in the absence of hip1Δ (Figure 4D). In addition, RT-qPCR revealed that in hip1Δ cells, Tf2 transcripts accumulate to levels that are greater than double that of the sre1-N strain in which Tf2 mobilization is dramatically increased (Figure 4E). Thus, unlike the sre1-N, abp1Δ, and set1Δ backgrounds, mobilization frequency does not correlate with expression in HIRA mutants (Figure 4F). One explanation of these results would be that HIRA is required for later stages of the Tf2 life cycle such as protein maturation. Similar to Tf1, Tf2 mRNA is translated as a single primary product and requires proteolytic processing by Tf2 protease to generate
mature proteins, including the IN (Levin et al. 1993; Hoff et al. 1998). To rule out that protein translation or post-translational processing of Tf2 proteins is not somehow impaired in hip1Δ cells, we monitored the levels of Tf2 IN by immunoblotting. Whereas Tf2 integrase is almost undetectable in WT cells, its level is dramatically increased in the hip1Δ mutant (Figure 4G). Therefore, the absence of elevated transposition in hip1Δ is unlikely due to defective post-transcriptional or post-translational processing of Tf2 products. Furthermore, deletion of hip1Δ did not reduce the elevated mobilization frequency observed in the sre1-N background (Figure 4B), which further supports the notion that loss of HIRA does not impair later stages in the Tf2 life cycle.

**HIRA is not required for the clustering of Tf2 elements**

Our data demonstrate that loss of transcriptional silencing is not necessarily sufficient to induce Tf2 mobilization and suggest that the propagation of these elements is subjected to additional host cell controls. A key candidate for one of these controls is higher-order chromatin organization, as these elements are physically clustered in the nucleus into structures termed Tf bodies (Cam et al. 2008). These structures are not necessary for silencing (Tanaka et al. 2012; Mikheyeva et al. 2014) but have been proposed to restrict the reintegration of Tf2 cDNA into the genome. Importantly, Tf2 clustering is lost in abp1Δ and set1Δ backgrounds (Cam et al. 2008; Lorenz et al. 2012) where expression and mobilization levels are well correlated. We therefore hypothesized that high levels of Tf2 mobilization requires both increased expression and clustering. This model predicts that Tf bodies will be lost in the presence of active Sre1 (sre1-N) but retained in cells lacking HIRA (hip1Δ). Therefore we used a FISH assay with a probe that spans the coding region of Tf2 to determine the status of clustering in hip1Δ and sre1-N backgrounds. As previously reported, the majority of WT cells displayed one or two Tf2 signals consistent with these elements being assembled into Tf bodies. In the sre1-N background the proportion of cells with three or more Tf signals was significantly increased (P < 0.001), indicating that Sre1-mediated activation of Tf2 transcription is accompanied by disruption to clustering (Figure 5A). Importantly, when hip1Δ cells were compared to WT, no significant increase (P > 0.05) in the proportion of cells with multiple Tf2 spots was observed (Figure 5B). Therefore, despite the very high levels of expression that are associated

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**Figure 4** Loss of HIRA-mediated silencing does not result in uncontrolled Tf2 element mobilization. (A) Midlog phase cells of the indicated strains were subjected to quantitative β-galactosidase assays. Mean values were determined from at least three independent assays and are scaled relative to WT. Error bars indicate ± SEM. (B) Deletion of hip1Δ results in only modest increase in Tf2 mobilization. The frequency of Tf2-12natAI mobilization was determined for the indicated strains by fluctuation analysis using the method of the median. Values were scaled relative to the WT. Error bars represent ± SEM. Data for sre1-N from Figure 2C are included for comparison. (C) Deletion of other HIRA complex genes does not stimulate Tf2 mobilization. Mobilization frequency was determined as described for B. (D) HIRA suppresses expression of the marked Tf2-12natAI element. RNA was prepared from the indicated strains and Tf2 mRNA levels were assayed by RT-qPCR and normalized to act1+ mRNA. Values are the mean of at least three biological repeats and error bars indicate ± SEM.

**P** 0.01, *P* 0.05, and ns (not significant) **P** > 0.05 (t-test). (F) Comparison of Tf2-lacZ expression with Tf2-12natAI mobilization frequency relative to WT levels in the indicated genetic backgrounds. (G) Increased levels of Tf2 IN in hip1Δ cells. Tf2 IN in WT and two hip1Δ strains were detected by immunoblotting. α-Tubulin (loading control) was detected with anti-tubulin Ab (tat-1).
with loss of HIRA, the sequestration of Tf2 elements into Tf bodies is retained. This suggests that clustering is important for restricting the propagation of Tf2 elements.

**Analysis of Set1 mutants suggests that Tf2 bodies restrict mobilization**

In order to further dissect the roles that spatial organization and silencing play in controlling the mobilization frequency of Tf2, we took advantage of some separation-of-function set1 mutant alleles. Previously it has been shown that Set1 utilizes distinct domains to repress Tf2 expression and maintain Tf bodies (Mikheyeva et al. 2014). For example, deletion of RNA recognition motif 2 (set1-RRM2Δ) results in partial loss of Tf2 repression but has only minimal disruption to Tf body integrity (Mikheyeva et al. 2014). Importantly, analysis of the Tf2-12natAI allele in a set1-RRM2Δ background revealed a low mobilization frequency that was not significantly increased relative to the WT (Figure 6, A and B). This is similar to the hip1Δ background, where an increase in expression in the presence of intact Tf bodies is apparently insufficient to elevate mobilization frequency. We next analyzed mobilization in a set1F-H3K4me background. This allele encodes a mutant protein that represses Tf2 expression but is unable to mediate either H3K4 methylation or Tf body maintenance (Mikheyeva et al. 2014). In this background, we found that mobilization frequency was modestly increased (threefold) relative to the WT, again suggesting that Tf bodies restrict the propagation of these elements. As predicted, a high mobilization frequency (ninefold relative to WT) was observed in the set1-SETΔ mutant, which lacks both transcriptional repression and Tf body maintenance functions (Mikheyeva et al. 2014). Taken together, these results indicate that Tf2 LTR retrotransposons are subjected to control at multiple levels and suggest that spatial organization functions to restrict their mobility. We therefore propose that the alleviation of transcriptional silencing and disruption of Tf bodies is necessary for high levels of element mobilization (Figure 7).

**Discussion**

Here we compared the impact of mutations upon both the expression and mobilization frequency of fission yeast Tf2 LTR retrotransposons. Our results indicate that host controls of the Tf2 life cycle operate at multiple levels and suggest that transcriptional silencing and higher-order chromatin organization cooperate to restrict the mobility of these elements.

That an increase in Tf2 mRNA is not necessarily accompanied by a proportional increase in mobilization indicates the existence of post-transcriptional controls. Post-transcriptional control of LTR retrotransposition has also been revealed in Arabidopsis as ablation of DNA methylation in met1 mutants does not increase the mobilization of retrotransposons despite their widespread transcriptional activation (Mirouze et al. 2009). Genetic analysis of the Évadé (EVD) copia-type LTR retrotransposon demonstrated that following transcriptional reactivation, subsequent steps in its lifecycle are suppressed by the plant-specific RNA polymerases IV/V and the histone methyltransferase KRYPTONITE. However while EVD mobilization is stimulated by the loss of these regulators, these mutations do not affect the mobilization of other potentially active retrotransposons (Mirouze et al. 2009). Therefore post-transcriptional controls of
retrotransposition may be individually tailored to specific elements.

Our analyses implicate subnuclear organization of Tf2s in the control of their mobilization. The fission yeast nucleus, similar to those of higher eukaryotes, is segregated into a variety of distinct chromosomal territories and domains and the importance of this organization genome function is becoming increasingly apparent (Nunez et al. 2009; Zhao et al. 2009; Tanizawa et al. 2010; Mizuguchi et al. 2014). Tf2 elements are subjected to a high degree of organization as they are clustered into bodies that are localized in close proximity to centromeres at the nuclear periphery (Cam et al. 2008; Tanaka et al. 2012). In these respects, Tf2 retrotransposons exhibit similarities with HIV-1 provirus in latently infected lymphocytes, which is also found associated with centromeric heterochromatin at the nuclear periphery (Dieudonne et al. 2009). In the case of HIV-1, nuclear positioning has been correlated to expression because transcriptional induction results in the loss of proviral association with heterochromatin, although localization at the nuclear periphery is retained (Dieudonne et al. 2009). Furthermore, it has been shown that inactive HIV-1 provirus is found in close proximity to PML bodies and that transcriptional activation requires displacement from these subnuclear structures (Lusic et al. 2013). However, in the case of Tf2 elements, transcriptional silencing can be separated from their spatial organization. The set1F-H3K4me mutant allele abolishes clustering but does not increase Tf2 expression (Mikhayeva et al. 2014). Also loss of Ku function (pku70Δ or pku80Δ) compromises interaction of Tf2 elements with centromeres and the nuclear periphery but does not impair the transcriptional silencing of these elements (Tanaka et al. 2012). Furthermore, we show here that Tf2 silencing can be circumvented without disruption to Tf bodies. We conclude therefore that the spatial organization of Tf2 elements does not restrict their mobility via an impact upon transcriptional silencing. Instead, we propose that Tf bodies restrict other steps in the retrotransposon life cycle (Figure 7).

Tf bodies have the potential to restrict the integration of cDNA into the genome, particularly as Tf2 elements prefer to mediate this step by homologous recombination into an existing element, a process which is termed “integration site recycling” (Hoff et al. 1998). Superficially, this may appear to be somewhat of a futile cycle. However, it has advantages in that it avoids integration into a region of the genome that is harmful to the host while still allowing element evolution (Hoff et al. 1998). Sequestration of these elements into Tf bodies may provide an environment that restricts their accessibility to suppress cDNA recombination. Consistent with this, abp1Δ cbh1Δ CENP-B double mutants, in which clustering is absent, have elevated levels of DNA recombination-associated Rad22 foci (homologous to S. cerevisiae Rad52) at LTRs (Zaratiegui et al. 2011). Tf bodies may also prevent recombination between different Tf2 elements, thereby suppressing potentially harmful chromosome rearrangements. Interestingly, it has been proposed that the compact chromatin conformation of S. cerevisiae Ty elements suppresses recombination hotspot activity and thus prevents potentially harmful exchange between these repeated sequences (Ben-Aroya et al. 2004). Nonetheless, Tf bodies could suppress alternative steps in the retrotransposon life cycle, such as RNA processing and export. There is clear precedent for the ability of chromatin structure to influence RNA processing steps (Mathieu and Boche 2014), and furthermore, links between nuclear bodies and a variety of RNA processing events have been well documented (Morimoto and Boerkoel 2013). Therefore, it is possible that Tf bodies could provide a molecular trap that restricts Tf2 mRNA processing and/or export. Arguing against this, levels of Tf2 IN were found to be markedly increased in the hip1Δ mutant, which retains Tf bodies. This suggests that Tf bodies do not prevent the export of Tf2 mRNA from the nucleus.

Transcriptional silencing is a common host cell response to transposable elements and is considered to be pivotal to controlling their activity. Our findings suggest that fission yeast cells can also restrict the mobilization of retroelements at a post-transcriptional level through epigenetic control of their nuclear organization. As such, it will be important to determine whether, and to what extent, the activity of retrotransposons in other systems is controlled by higher-order organization of chromatin.

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Restriction of Retrotransposon Mobilization in *Schizosaccharomyces pombe* by Transcriptional Silencing and Higher-Order Chromatin Organization

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Table S1. Strains used in this study.

| Strain   | Genotype                                      | Ref/Source                        |
|----------|-----------------------------------------------|-----------------------------------|
| HM19     | h- ade6-M210 leu1-32 ura4-D18 his3-D1 Tf2-12natAI | This study                        |
| HM20     | h- ade6-M210 leu1-32 ura4-D18 his3-D1 Tf2-12natAI | This study                        |
| HM284    | h- ade6-M210 leu1-32 ura4-D18 rad51::ura4+ his3-D1 Tf2-12natAI | This study                        |
| HM136    | h- ade6-M216 leu1-32 ura4-D18 Tf2-lacZ(ura4+)   | (ANDERSON et al. 2009)            |
| HM246    | h- ade6-M210 leu1-32 ura4-D18 his3-D1 Tf2-12natAI sre1-N(kanMX) | This study                        |
| HM294    | h ade6 leu1-32 ura4-D18 Tf2-lacZ(ura4+) sre1-N(kanMX) | This study                        |
| SW898    | h ade6 leu1-32 ura4-D18 Tf2-lacZ(ura4+) set1::kanMX | This study                        |
| SW904    | h ade6 leu1-32 ura4-D18 his3-D1 Tf2-12natAI set1::kanMX | This study                        |
| HM216    | h ade6 leu1-32 ura4-D18 abp1::ura4+ Tf2-12natAI | This study                        |
| HM316    | h ade6 leu1-32 ura4-D18 abp1::ura4+ Tf2-lacZ(ura4+) | This study                        |
| HM259    | h ade6-M210 leu1-32 ura4-D18 Tf2-12natAI clr6-1 | This study                        |
| JW19     | h- ade6-M216 leu1-32 ura4-D18 Tf2-lacZ(ura4+) hip1::ura4+ | (ANDERSON et al. 2009)            |
| HM55     | h ade6 leu1-32 ura4-D18 his3-D1 Tf2-12natAI hip1::ura4+ | This study                        |
| HM57     | h ade6 leu1-32 ura4-D18 his3-D1 Tf2-12natAI hip1::ura4+ | This study                        |
| HM307    | h ade6 leu1-32 ura4-D18 hip1::ura4+ Tf2-12natAI sre1-N(kanMX) | This study                        |
| HM538    | h ade6 leu1-32 ura4-D18 his3-D1 Tf2-12natAI slm9::ura4+ | This study                        |
| HM540    | h ade6 leu1-32 ura4-D18 his3-D1 Tf2-12natAI hip3::ura4+ | This study                        |
| HM542    | h ade6 leu1-32 ura4-D18 his3-D1 Tf2-12natAI hip4::ura4+ | This study                        |
| SPHC1051 | mat1-msto ade6-M216 leu1-32 ura4-DS/E his2 otrR1::ura4 set1::kanMX | (LORENZ et al. 2012)              |
| SPHC88   | mat1-msto ade6-M210 leu1-32 ura4-DS/E otrR1::ura4 his2 | Lab stock                        |
| PEY675   | h- ade6-M210 leu1-32 ura4-D18 his3-D1 sre1-N(kanMX) | (LEE et al. 2009)                 |
| SW137    | h- ade6-M210 leu1-32 ura4-D18 hip1::ura4+       | (BLACKWELL et al. 2004)           |
| SW943    | h ade6-M210 leu1-32 ura4-D18 his lys7-2? FLAGset1-RRM2Δ Tf2-12natAI | This study                        |
| SW941    | h- ade6-M210 leu1-32 ura4-D18 his otrR1::ura4 set1-3xFLAG(kanMX) Tf2-12natAI | This study                        |
| SW947    | h/mat1-msto ade6-M210 leu1-32 ura4-D18 his lys7-2? FLAG-set1-SETAΔ Tf2-12natAI | This study                        |
| SW975    | h- ade6-M210 leu1-32 ura4-D18 his3-D1 Tf2-12natAI dcr1::kanMX | This study                        |

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