Current Biology 23, 345–350, February 18, 2013 ©2013 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2013.01.030

Report

A Matrix Protein Silences Transposons and Repeats through Interaction with Retinoblastoma-Associated Proteins

Yifeng Xu,[1] Yizhong Wang,[1,2] Hume Stroud,[3] Xiaofeng Gu,[1] Bo Sun,[1] Eng-Seng Gan,[1] Kian-Hong Ng,[1] Steven E. Jacobsen,[3,4] Yuehui He,[1,2,*] and Toshiro Ito[1,2,*]
[1]Temasek Life Sciences Laboratory (TLL), 1 Research Link, National University of Singapore, Singapore 117604, Singapore
[2]Department of Biological Sciences, Faculty of Science, National University of Singapore, 10 Science Drive 4, 117543, Singapore
[3]Department of Molecular, Cell and Developmental Biology
[4]Howard Hughes Medical Institute
University of California, Los Angeles, Los Angeles, CA 90095, USA

Summary

Epigenetic regulation helps to maintain genomic integrity by suppressing transposable elements (TEs) and also controls key developmental processes, such as flowering time [1–3]. To prevent TEs from causing rearrangements and mutations, TE and TE-like repetitive DNA sequences are usually methylated, whereas histones are hypoacetylated and methylated on specific residues (e.g., H3 lysine 9 dimethylation [H3K9me2]) [4, 5]. TEs and repeats can also attenuate gene expression [2, 6–8]. However, how various histone modifiers are recruited to target loci is not well understood. Here we show that knockdown of the nuclear matrix protein with AT-hook DNA binding motifs [9–11] TRANSPOSABLE ELEMENT SILENCING VIA AT-HOOK (TEK) in *Arabidopsis* Landsberg *erecta* results in robust activation of various TEs, the TE-like repeat-containing floral repressor genes *FLOWERING LOCUS C* (*FLC*) and *FWA* [1, 2, 12]. This derepression is associated with chromatin conformational changes, increased histone acetylation, reduced H3K9me2, and even TE transposition. TEK directly binds to an *FLC*-repressive regulatory region and the silencing repeats of *FWA* and associates with *Arabidopsis* homologs of the Retinoblastoma-associated protein 46/48, FVE and MSI5, which mediate histone deacetylation [13, 14]. We propose that the nuclear matrix protein TEK acts in the maintenance of genome integrity by silencing TE and repeat-containing genes.

Results and Discussion

TEK Knockdown Leads to Late Flowering

Matrix proteins with AT-hook DNA binding motifs bind to the AT-rich nuclear matrix attachment regions, possibly affecting the epigenetic state of target chromatin in animals and plants [15–17]. Some AT-hook proteins are known to regulate tissue-specific gene expression [15, 16] but very little is known about their functional mechanisms, especially in gene silencing. Most of the *Arabidopsis* AT-hook DNA binding proteins are dominantly expressed in root, but *AHL16* (*At2g42940*, we renamed it *TEK*) is preferentially expressed in the inflorescence meristem and young floral buds, as well as in seedling-stage vegetative meristems (the *Arabidopsis* eFP browser http://bbc.botany.utoronto.ca/efp; Figures 1A–1D and see Figure S1 available online). To study *TEK* functions, we created two artificial microRNA (amiRNA) constructs [18], *35S::amiTEKa* and *35S::amiTEKb*, to target the *TEK* coding and 3′ untranslated regions, respectively (Figure 1E). Transgenic lines for both constructs with strongly reduced *TEK* levels (20%–40% compared to the wild-type) showed late-flowering phenotypes in the Landsberg *erecta* (L*er*) ecotype background (Figures 1F–1I and S2A and S2B). About 40% of T_1 *35S::amiTEKb* plants showed extremely late flowering (Figures 1F). RNA levels of control genes including the closest homolog *AHL28* were unaffected (Figure S2C). Many T1 *amiTEK* transgenic plants also showed altered phyllotaxis, extra cauline leaves of larger size, and reduced fertility, in agreement with the expression pattern of *TEK* in the inflorescence meristem and reproductive organs (Figures 1D and 1J; data not shown) [19]. It is notable that none of the *35S::amiTEK* plants in the Colombia (Col) background showed late flowering (data not shown), suggesting that *TEK* knockdown may have accession-specific effects. We also identified *T-DNA* insertion mutants (*tek-1*, *tek-2*, and *tek-3*) in Col or Wassilewskija (Ws) (Figure S2D). These mutants were sterile, but they showed no obvious defects in flowering time (data not shown). Next, we confirmed that the late-flowering phenotype is accession dependent by backcrossing *tek-1* (in the Col background) into L*er*. Some homozygous plants started to exhibit late flowering after the fourth backcross (Figure S2E).

TEK Directly Controls *FLC* Expression and Chromatin Conformation

To examine what genes cause the delayed flowering in the *TEK* knockdown plants, we examined transcript levels of flowering regulators in stable and fertile *amiTEK* lines (they are called "*amiTEK*") (Figures 1K–1M and S2F–S2J). We found that *FLC*, a central floral repressor (reviewed in [20]), was dramatically upregulated (Figure 1K), whereas the levels of *FLC* downstream flowering integrators, including *AGL24*, *SOC1*, and *FT* [20], were moderately decreased (Figures 1L–1M and S2F). Furthermore, transcript levels of the *FLC* upstream factors such as the putative histone H3 lysine 4 demethylase FLD, FVE, and FRI LIKE 1 (FRL1) were not significantly changed (Figures S2G–S2J), indicating direct regulation of *FLC* by TEK.

To test whether TEK directly binds to *FLC* chromatin, we performed chromatin immunoprecipitation (ChIP) using the lines expressing the functional TEK with epitope tags driven by a ubiquitous promoter or the native *TEK* promoter. Enrichment with a primer set spanning 5′ end of the first intron (region c), essential for *FLC* silencing [21], was detected (Figures 2A, 2B, S3A, and S3B). Because *TEK* encodes an AT-hook DNA binding matrix protein, we next tested whether nuclear matrix association of *FLC* is affected in *amiTEK*. DNA attached to nuclear matrix is enriched by washing purified nuclei with high salt buffer [16]. We detected a dramatic increase in the ratio of free DNA to nuclear matrix-attached DNA in *amiTEK* compared to wild-type (Figures 2A and 2C). These results

*Correspondence: dbshy@nus.edu.sg (Y.H.), itot@tll.org.sg (T.I.)

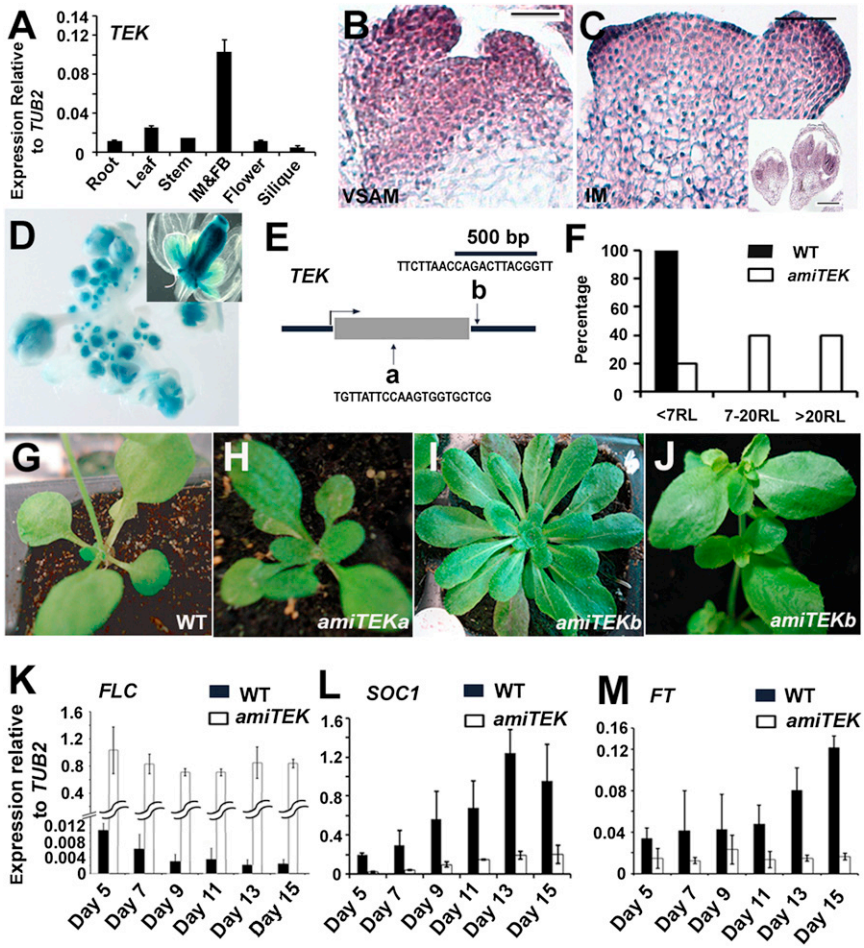

Figure 1. *TEK* Knockdown Leads to Significant *FLC* Derepression and Late Flowering

(A) The relative expression of *TEK* to *TUBULIN2 (TUB2)* in root, leaf, stem, inflorescence meristem, and young flower bud (IM&FB), flower, and silique. (B and C) In situ hybridization of *TEK* in the vegetative shoot apical meristem (VSAM) 10 days after germination (DAG 10) (B), the inflorescence meristem (IM) (C), and young floral buds (inset). Scale bars represent 25 μm for (B) and 50 μm for (C). (D) Spatial expression pattern of *pTEK*::*TEK-GUS* (β-glucuronidase) in the reproductive tissues. *TEK* is highly expressed in the whole inflorescence and ovules and anthers of a developing flower (inset). (E) The locations and target sequences of the artificial *TEK* knockdown constructs a and b. (F–J) Knockdown of *TEK* by *amiTEKa* and *amiTEKb* caused late flowering. Distribution of late-flowering phenotypes of 80 T_1 lines with *amiTEKb* under continuous light growth condition is shown by the number of rosette leaves (RL) (F), wild-type L*er* plant (G), a *35S::amiTEKa* plant (H), and *35S::amiTEKb* plants (I) and (J). (K–M) Expression analyses of *FLC* (K), *SOC1* (L), and *FT* (M) relative to *TUB2* in DAG 5, 7, 9, 11, 13, and 15 in wild-type L*er* (WT) and *amiTEK* plants. Error bars represent SD based on three biological replicates.

suggest that TEK is necessary for the association of *FLC* chromatin with the nuclear matrix for silencing.

TEK Knockdown Leads to Reduced H3K9me2 and Increased H3 Acetylation

Next, we compared histone modifications in wild-type and *amiTEK* by ChIP. We found that in wild-type seedlings, the repressive mark H3K9me2 was enriched in *FLC*, especially at the TEK binding site (region c) and 3′ region of the intron 1 (region e) (Figures 2A and 2D). This enrichment was abolished in *amiTEK* (Figures 2D and S3C). In contrast, the activation mark H3 acetylation (H3Ac) was increased in *amiTEK* compared with wild-type (Figures 2E, S3D, and S3E). Notably, another repressive mark, histone H3 lysine 27 trimethylation (H3K27me3), was not primarily changed in *amiTEK* (Figure 2F and S3F). Together, these data suggest that TEK may function through H3K9 dimethylation and histone deacetylation.

TEK Knockdown Leads to Robust Derepression of TEs and Even Transposition of the *Mutator*-like TE from L*er FLC*

We performed global transcriptional analyses in *amiTEK* using the *Arabidopsis* oligonucleotide microarray (NimbleGen, Roche). We detected 1,209 genes including *FLC* upregulated and 416 genes downregulated in *amiTEK* seedlings compared with wild-type ($p < 0.05$) (Figure 3A, Table S1, NCBI Gene Expression Omnibus GSE39158). Among the upregulated genes, around 69% were TEs. Drastic upregulation of various retrotransposons and DNA transposons [22–24] was confirmed in two independent *amiTEK* lines (Figure 3B).

To further confirm TE activation upon the loss of *TEK* function, we performed RNA-sequencing using the *tek-1* inflorescences showing late-flowering phenotypes. It showed that 75 TEs were upregulated over 4-fold compared to the wild-type L*er* ($p < 0.05$ cutoff) (Table S2). We also detected an overlap of upregulated protein-coding genes between *amiTEK* and the introgressed *tek-1* (Figure S4A, Table S3). Moderate activation of several representative TEs as well as *FLC* was further confirmed in the seedling of introgressed *tek-1* homozygous lines (Figure S4B) but not in the original Col background (data not shown). These show that TEK is essential for TE silencing in L*er*.

The L*er FLC* allele contains a *Mutator*-like insertion in the first intron, which silences *FLC* expression [2, 6] (Figure 3C). The significant derepression of L*er FLC* in *amiTEK* prompted us to check whether the TE insertion was still present. Strikingly, it was lost in four randomly picked T_1 lines with late-flowering phenotypes (Figures 3C and 3D). Sequencing of the excision sites of 12 independent lines (from two transformation experiments) showed that all lines have the exact same sequences as Col (data not shown), suggesting that the excision happened multiple times at the same position. In rice, one *Mutator*-like element has been reported to be excised without footprint [25]. Thus, the precise excision is likely to be a common feature in *Arabidopsis* and rice. Furthermore, the excision and transposition of the *Mutator*-like TE and two other DNA transposons *CACTA* and *hAT* were confirmed in two independent lines by genomic Southern blots (Figure 3E, marked as black arrows; Figure S4C). TEK binding to *FLC* was detected at a region about 1 kb upstream of the *Mutator*-like TE in both Col and L*er*

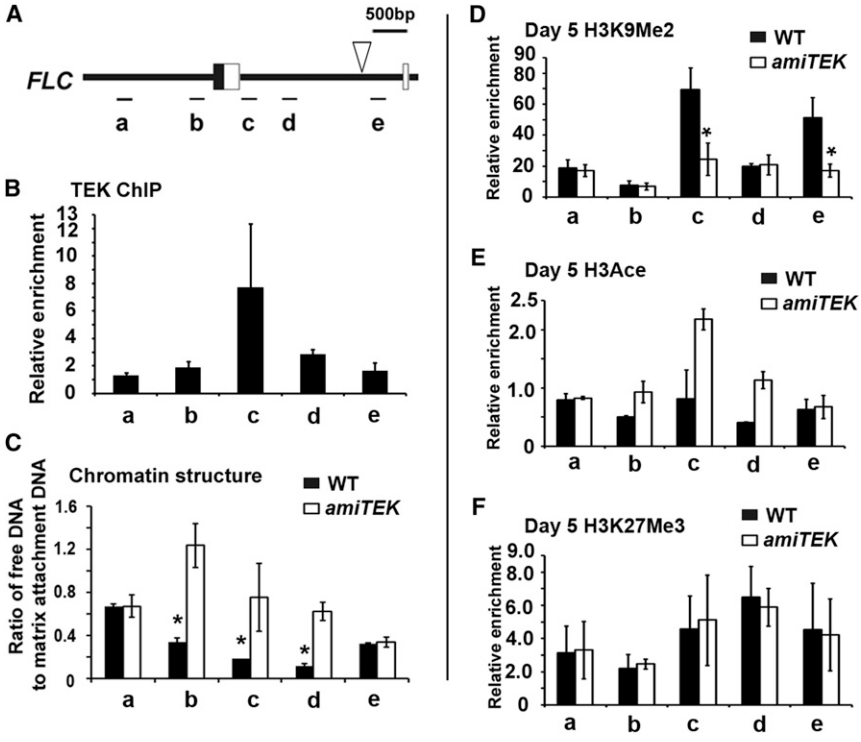

Figure 2. TEK Binds to *FLC* Chromatin, Mediates Histone Modification, and Maintains Chromatin Structure at the *FLC* Locus

(A) Schematic structure of the *FLC* locus showing the 5′ untranslated region (black box) and first two exons (white boxes). Letters a to e in (A)–(F) represent the amplicons examined by qPCR. White triangle, 1.2 kb TE insertion in L*er*. (B) Binding of TEK to *FLC* analyzed by ChIP using *pTEK::TEK-YFP* inflorescences. (C) *TEK* knockdown changed the matrix association of *FLC*. (D–F) Relative fold enrichment of epigenetic marks at *FLC*, H3K9me2 (D), H3 acetylation (E), and H3K27me3 (F) using the aerial parts of DAG 5 plants. Error bars represent SD based on three biological replicates in (B)–(D) and (F) and on two biological replicates in (E). Asterisks indicate statistically significant differences (paired Student's t test, $p < 0.05$) between samples in WT and *amiTEK*.

(Figures 2B and S3B), suggesting that TEK may be involved in *FLC* silencing, independently of TE silencing. Thus, *FLC* activation may have contributed to the TE transposition and vice versa.

The Repeat-Containing *FWA* Gene Is Also Derepressed and Contributes to Late-Flowering in *amiTEK*

We also found that the expression of *FWA*, which contains two tandem repeats of a *SINE*-like element near its transcription start site [8], was ectopically induced in *amiTEK* (Figures S4D–S4F). To see whether the late-flowering phenotype is attributed to ectopic expression of both *FLC* and *FWA*, we performed vernalization treatment (an extended period of cold exposure) on *amiTEK* because vernalization represses *FLC*, but not *FWA* (Figures S4E and S4F) [26]. The vernalization-treated *amiTEK* flowered earlier than those without treatment but still flowered later than wild-type (Figure S4D). These suggest that both *FLC* and *FWA* contribute to late flowering in *amiTEK*.

Because that ectopic expression of *FWA* in vegetative tissues and derepression of TEs are reportedly associated with loss of DNA methylation [12], we used bisulfite sequencing to examine DNA methylation of *FWA*. Methylation at CG, CNG, and CHH was reduced in the tandem repeats of *FWA* in *amiTEK* (Figure 3F). However, these reductions are not as great as those found in the *epi* mutant allele of *fwa*, in which DNA methylation was almost abolished [12]. At *AtMu1*, which is related to the *Mutator*-like TE at L*er FLC*, the percentages of methylated CG, CNG, and CHH were also moderately decreased (Figure S4G).

Next, we examined the histone modification state at *FWA*. The level of H3K9me2 was reduced in the tandem repeats (region b) of *FWA* in *amiTEK* (Figures S4H and S4I). The acetylation level was increased significantly in region b and moderately in region a (Figures 3G and 3H), whereas the H3K27me3 level did not show any obvious change (Figure S4J). The change in histone acetylation at *FWA* appears greater than the change in DNA methylation in *amiTEK*, suggesting that histone deacetylation may play a greater role in *FWA* silencing. The ChIP assays using *pTEK::TEK-YFP* confirmed that TEK directly binds to the repeated sequences of *FWA* (Figure 3I), suggesting that *FWA* is a direct TEK target.

TEK Associates with FVE and Its Homolog MSI5, Components of HDAC Corepressor Complexes

To understand the causal link between TEK and histone modifications, we performed yeast two-hybrid assays to examine the interaction of TEK with various histone-modification factors, including HDAC, FVE, MSI5, and Polycomb group proteins. We found that TEK interacted with FVE and MSI5, but not with others (Figure 4A and data not shown). The interactions were further confirmed in plant cells by bimolecular fluorescence complementation (BiFC). Fluorescence was observed in the nuclei of onion epidermal cells only when TEK and FVE or TEK and MSI5 constructs were coinjected (Figure 4B). Moreover, we carried out coimmunoprecipitation experiments to confirm the in vivo association of TEK with FVE/MSI5 using the seedlings expressing the TEK-YFP and FVE-FLAG/MSI5-FLAG. We found that anti-FLAG (recognizing FVE-FLAG and MSI5-FLAG) coimmuoprecipitated TEK-YFP from the seedlings (Figure 4C). Together, these show that TEK is in a complex with FVE/MSI5 in *Arabidopsis*.

Because both FVE and MSI5, *Arabidopsis* homologs of the mammalian Retinoblastoma-associated protein 46/48, are components of histone deacetylation complexes that silence *FLC*, TEs (e.g., *AtMu1*) and repetitive sequence-containing loci (e.g., *FWA*) [14], the TEK-FVE/MSI5 complex is likely to bind to TEs. However, we were unable to detect significant ChIP enrichments of TEK at TEs (data not shown). This might be due to weak binding abilities of AT-hook proteins. In *Brassica*, it has been shown that SINE-like repeats are highly associated with nuclear matrix [27]. Taken together, we propose that the nuclear matrix protein TEK associates with the FVE/MSI5 complex and binds to various target sites of

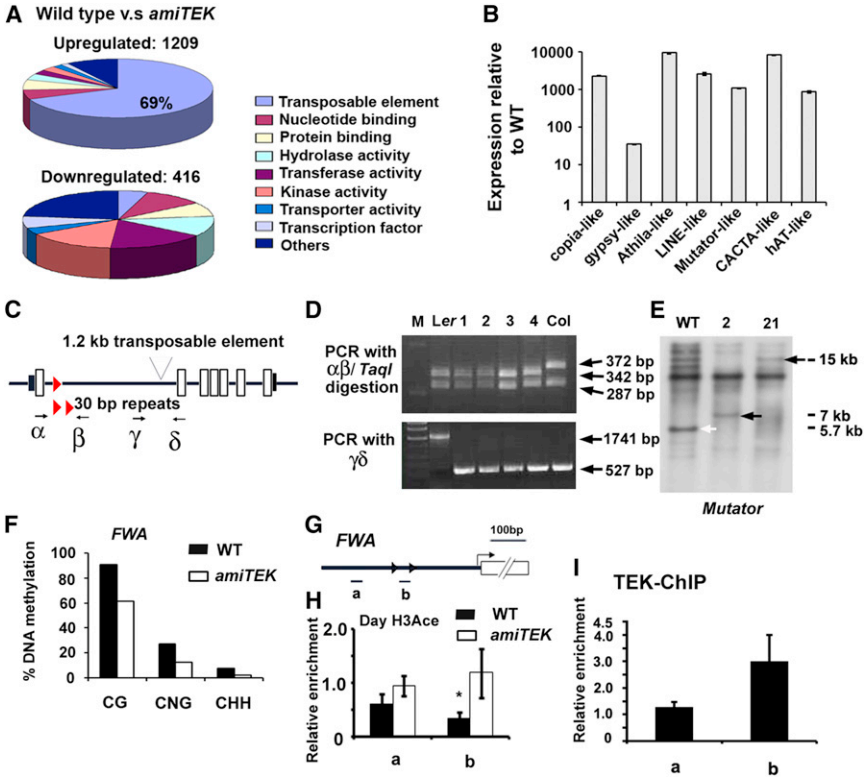

Figure 3. *TEK* Knockdown Causes TE Derepression and TE Transposition from L*er FLC*

(A) Pie chart of microarray results comparing *amiTEK* and wild-type L*er* plants at DAG 5. (B) The relative ratio of TE transcript levels in *amiTEK* and wild-type representative. TEs: *Copia-like (AT5G43800)*, *gypsy-like (AT5G33050)*, *Athila-like (AT5G32306)*, *LINE-like (AT3G43436)*, *Mutator-like (AT1G33460)*, *CACTA-like (AT5G45082)*, and *hAT-like (AT2G05700)*. (C and D) The *Mutator*-like element in the intron 1 of L*er FLC* was lost in *amiTEK*. Black and white boxes, untranslated and coding regions, respectively. Arrowheads, the 30 bp repeat in the intron 1 (one in L*er* and two in Col). In four randomly picked late-flowering plants in the L*er* background (1–4), the 1.2 kb TE was absent (D). (E) A genomic Southern blot showed that the *Mutator*-like TE in *FLC* (white arrow) was excised and translocated in *amiTEK* (lines 2 and 21, black arrows). (F) Bisulfite sequencing showed that cytosine methylation levels of CG, CHG, and CHH at *FWA* were decreased in *amiTEK*. (G) Schematic structure of *FWA*. Arrowheads, the *SINE*-like direct repeats. Letters a and b show regions tested in (H) and (I). (H) H3 acetylation levels were increased in *amiTEK*. Asterisk indicates statistically significant difference (paired Student's t test, $p < 0.05$) between samples. (I) Binding of TEK to *FWA* by ChIP using *pTEK::TEK-YFP* inflorescences. Error bars represent SD based on three biological replicates.

TEs and repeat-containing genes, leading to histone deacetylation and, thus, gene silencing (Figure 4D).

We compared the upregulated genes in *amiTEK* with those in the mutants for the DNA methyltransferase MET1 and the histone deacetylase HDA6 [28–30]. Significant overlap of genes silenced by TEK, MET1, and HDA6 was detected, including some of siRNA-directed DNA methylation targets (Figures S4K and S4L). These indicate the cooperative action of DNA methylation, histone deacetylation, and TEK. In addition, HDA6 can directly interact with MET1 [31], indicating that TEK may act as a part of large protein complexes including histone deacetylase as well as DNA methyltransferase.

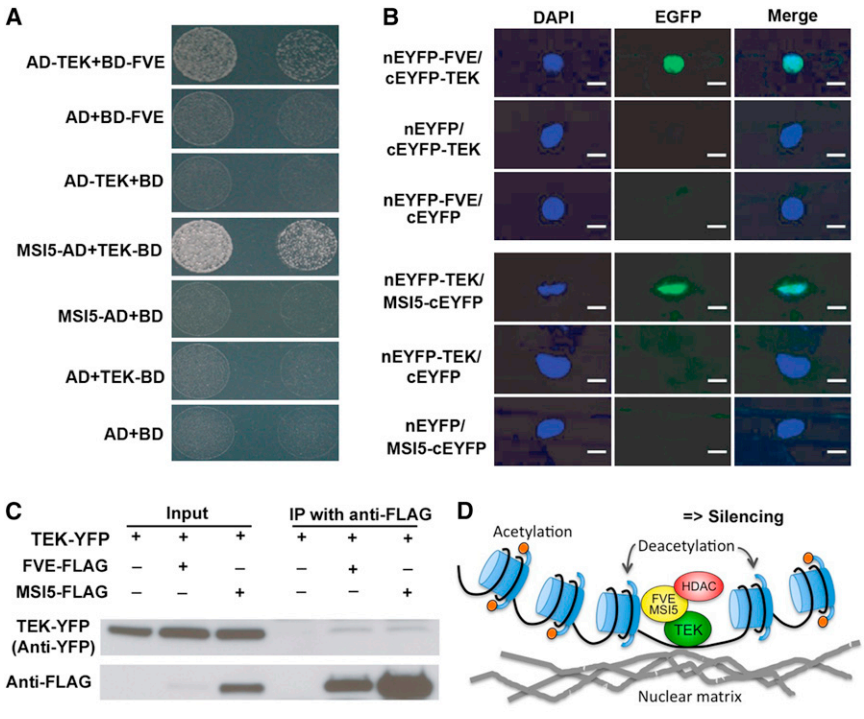

Figure 4. TEK Directly Associates with FVE and MSI5

(A) Interaction of TEK with FVE and MSI5 analyzed by yeast two-hybrid assay. Full-length TEK, FVE, or MSI5 was fused to GAL4 activation (AD) and/or DNA binding domains (BDs), respectively. Yeast colonies harboring these fusion constructs and/or empty vectors, as indicated, were grown on selective media. Yeast growth was detected only when the combinations of TEK and FVE or TEK and MSI5 were cotransformed. (B) BiFC analysis of TEK association with FVE and MSI5 in onion epidermal cells. Green signal indicates the binding of TEK with FVE or MSI5 in the nuclei. DAPI (4',6-diamidino-2-phenylindole) staining indicates nuclei. Scale bar represents 20 μm. (C) Coimmunoprecipitation of TEK with FVE and MSI5 in seedlings. Total proteins extracted from the *TEK-YFP* line (a negative control), F_1 of the doubly hemizygous *TEK-YFP* and *FVE-FLAG*, and F_1 of *TEK-YFP* and *MSI5-FLAG* were immunoprecipitated with anti-FLAG agarose beads. The TEK-YFP protein was specifically detected in the anti-FLAG precipitates from the F_1 seedlings expressing FVE-FLAG or MSI5-FLAG. (D) A model of TEK function. TEK binds to specific targets and forms a protein complex with FVE/MSI5, which participates in histone deacetylation. Deacetylation of the target locus leads to transcriptional silencing.

In summary, our data show that *TEK* acts in the maintenance of genomic integrity by silencing TEs and repeat-containing genes through epigenetic machinery. In *tek* insertional mutants in the Col and WS backgrounds, *FLC* was not derepressed, in contrast to *FLC* derepression in the *fve* mutant in the Col background [13]. The different ecotypic effects of *tek* mutations and *amiTEK* in Col and L*er* suggest that these two ecotypes may have different susceptibility to the loss of TEK activities. This may be due to presence of redundant genes that can substitute for *TEK*, downstream effectors of TEK or a parallel pathway for silencing in Col and WS (but not in L*er*). Such accession-specific effects have been reported in epigenetic regulation including differentially expressed small RNAs and genome imprinting in *Arabidopsis* [23, 32]. Further studies are needed to identify the genetic modifier(s) in different ecotypes.

Supplemental Information

Supplemental Information includes four figures, four tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.01.030.

Acknowledgments

We thank Arabidopsis TAIR (http://arabidopsis.org) for information and materials and also H. Yu and F. Berger for valuable comments on the manuscript. This work was supported by research grants to T.I. from Temasek Life Sciences Laboratory (TLL), the National Research Foundation Singapore under its Competitive Research Programme (CRP Award NRF-CRP001-108), and PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama, Japan. The work in Y.H.'s laboratory was supported by a grant from the Singapore Ministry of Education (AcRF Tier 2; T207B3105), CRP Award NRF-CRP001-108, and TLL. The research in the S.E.J. laboratory was supported by National Science Foundation Grant MCB-1121245. Illumina sequencing was performed at the UCLA BSCRC BioSequencing Core Facility. H.S. was supported by a Fred Eiserling and Judith Lengyel Graduate Doctorate Fellowship. S.E.J. is an Investigator of the Howard Hughes Medical Institute.

Received: August 4, 2012
Revised: December 12, 2012
Accepted: January 11, 2013
Published: February 7, 2013

References

1. Liu, J., He, Y., Amasino, R., and Chen, X. (2004). siRNAs targeting an intronic transposon in the regulation of natural flowering behavior in Arabidopsis. Genes Dev. *18*, 2873–2878.
2. Gazzani, S., Gendall, A.R., Lister, C., and Dean, C. (2003). Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. Plant Physiol. *132*, 1107–1114.
3. Law, J.A., and Jacobsen, S.E. (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat. Rev. Genet. *11*, 204–220.
4. Zaratiegui, M., Irvine, D.V., and Martienssen, R.A. (2007). Noncoding RNAs and gene silencing. Cell *128*, 763–776.
5. Saze, H., and Kakutani, T. (2011). Differentiation of epigenetic modifications between transposons and genes. Curr. Opin. Plant Biol. *14*, 81–87.
6. Michaels, S.D., He, Y., Scortecci, K.C., and Amasino, R.M. (2003). Attenuation of FLOWERING LOCUS C activity as a mechanism for the evolution of summer-annual flowering behavior in Arabidopsis. Proc. Natl. Acad. Sci. USA *100*, 10102–10107.
7. Lippman, Z., Gendrel, A.V., Black, M., Vaughn, M.W., Dedhia, N., McCombie, W.R., Lavine, K., Mittal, V., May, B., Kasschau, K.D., et al. (2004). Role of transposable elements in heterochromatin and epigenetic control. Nature *430*, 471–476.
8. Kinoshita, Y., Saze, H., Kinoshita, T., Miura, A., Soppe, W.J., Koornneef, M., and Kakutani, T. (2007). Control of FWA gene silencing in Arabidopsis thaliana by SINE-related direct repeats. Plant J. *49*, 38–45.
9. Reeves, R., and Nissen, M.S. (1990). The A.T-DNA-binding domain of mammalian high mobility group I chromosomal proteins. A novel peptide motif for recognizing DNA structure. J. Biol. Chem. *265*, 8573–8582.
10. Yasui, D., Miyano, M., Cai, S.T., Varga-Weisz, P., and Kohwi-Shigematsu, T. (2002). SATB1 targets chromatin remodelling to regulate genes over long distances. Nature *419*, 641–645.
11. Cai, S., Han, H.J., and Kohwi-Shigematsu, T. (2003). Tissue-specific nuclear architecture and gene expression regulated by SATB1. Nat. Genet. *34*, 42–51.
12. Soppe, W.J., Jacobsen, S.E., Alonso-Blanco, C., Jackson, J.P., Kakutani, T., Koornneef, M., and Peeters, A.J. (2000). The late flowering phenotype of fwa mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. Mol. Cell *6*, 791–802.
13. Ausín, I., Alonso-Blanco, C., Jarillo, J.A., Ruiz-García, L., and Martínez-Zapater, J.M. (2004). Regulation of flowering time by FVE, a retinoblastoma-associated protein. Nat. Genet. *36*, 162–166.
14. Gu, X., Jiang, D., Yang, W., Jacob, Y., Michaels, S.D., and He, Y. (2011). Arabidopsis homologs of retinoblastoma-associated protein 46/48 associate with a histone deacetylase to act redundantly in chromatin silencing. PLoS Genet. *7*, e1002366.
15. Han, H.J., Russo, J., Kohwi, Y., and Kohwi-Shigematsu, T. (2008). SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. Nature *452*, 187–193.
16. Ng, K.H., Yu, H., and Ito, T. (2009). AGAMOUS controls GIANT KILLER, a multifunctional chromatin modifier in reproductive organ patterning and differentiation. PLoS Biol. *7*, e1000251.
17. Yun, J., Kim, Y.S., Jung, J.H., Seo, P.J., and Park, C.M. (2012). The AT-hook motif-containing protein AHL22 regulates flowering initiation by modifying FLOWERING LOCUS T chromatin in Arabidopsis. J. Biol. Chem. *287*, 15307–15316.
18. Schwab, R., Ossowski, S., Riester, M., Warthmann, N., and Weigel, D. (2006). Highly specific gene silencing by artificial microRNAs in Arabidopsis. Plant Cell *18*, 1121–1133.
19. Alves-Ferreira, M., Wellmer, F., Banhara, A., Kumar, V., Riechmann, J.L., and Meyerowitz, E.M. (2007). Global expression profiling applied to the analysis of Arabidopsis stamen development. Plant Physiol. *145*, 747–762.
20. Amasino, R. (2010). Seasonal and developmental timing of flowering. Plant J. *61*, 1001–1013.
21. He, Y., Michaels, S.D., and Amasino, R.M. (2003). Regulation of flowering time by histone acetylation in Arabidopsis. Science *302*, 1751–1754.
22. Singer, T., Yordan, C., and Martienssen, R.A. (2001). Robertson's Mutator transposons in A. thaliana are regulated by the chromatin-remodeling gene Decrease in DNA Methylation (DDM1). Genes Dev. *15*, 591–602.
23. Zhai, J.X., Liu, J., Liu, B., Li, P.C., Meyers, B.C., Chen, X.M., and Cao, X.F. (2008). Small RNA-directed epigenetic natural variation in Arabidopsis thaliana. PLoS Genet. *4*, e1000056.
24. Tsukahara, S., Kobayashi, A., Kawabe, A., Mathieu, O., Miura, A., and Kakutani, T. (2009). Bursts of retrotransposition reproduced in Arabidopsis. Nature *461*, 423–426.
25. Gao, D. (2012). Identification of an active Mutator-like element (MULE) in rice (Oryza sativa). Mol. Genet. Genomics *287*, 261–271.
26. Johanson, U., West, J., Lister, C., Michaels, S., Amasino, R., and Dean, C. (2000). Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. Science *290*, 344–347.
27. Tikhonov, A.P., Lavie, L., Tatout, C., Bennetzen, J.L., Avramova, Z., and Deragon, J.M. (2001). Target sites for SINE integration in Brassica genomes display nuclear matrix binding activity. Chromosome Res. *9*, 325–337.
28. Aufsatz, W., Mette, M.F., van der Winden, J., Matzke, M., and Matzke, A.J. (2002). HDA6, a putative histone deacetylase needed to enhance DNA methylation induced by double-stranded RNA. EMBO J. *21*, 6832–6841.
29. Zilberman, D., Gehring, M., Tran, R.K., Ballinger, T., and Henikoff, S. (2007). Genome-wide analysis of Arabidopsis thaliana DNA methylation uncovers an interdependence between methylation and transcription. Nat. Genet. *39*, 61–69.
30. To, T.K., Kim, J.M., Matsui, A., Kurihara, Y., Morosawa, T., Ishida, J., Tanaka, M., Endo, T., Kakutani, T., Toyoda, T., et al. (2011). Arabidopsis

HDA6 regulates locus-directed heterochromatin silencing in cooperation with MET1. PLoS Genet. *7*, e1002055.

31. Liu, X., Yu, C.W., Duan, J., Luo, M., Wang, K., Tian, G., Cui, Y., and Wu, K. (2012). HDA6 directly interacts with DNA methyltransferase MET1 and maintains transposable element silencing in Arabidopsis. Plant Physiol. *158*, 119–129.
32. Gehring, M., Bubb, K.L., and Henikoff, S. (2009). Extensive demethylation of repetitive elements during seed development underlies gene imprinting. Science *324*, 1447–1451.