Flowering and genome integrity control by a nuclear matrix protein in Arabidopsis

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The nuclear matrix is a supporting structural component that remains inside the nucleus after removal of basic proteins and histones. The interactions between chromatin and the nuclear matrix occur through AT-rich sequences of high affinity, named as matrix or scaffold attachment regions (MARs or SARs) (reviewed in ref. 1). MARs are commonly found at the boundaries of transcription units or close to regulatory cis-elements, and function in several biological processes such as forming higher order chromosome structures, regulating gene expression and facilitating DNA replications. Not all potential MARs are associated with the nuclear matrix at all times; in fact, MARs are dynamically anchored to the nuclear matrix by MAR-binding proteins in cell-type and/or cell-cycle-dependent manners. AT-hook DNA-binding proteins are a kind of MAR-binding proteins and have a variable number of AT-hook motifs, which are characterized by a typical sequence pattern centered around a highly conserved tripeptide of Gly-Arg-Pro (GRP). AT-hook motifs are able to bind to minor grooves of stretches of MARs in a non-strictly sequence-specific manner, while common transcription factors usually bind to the major grooves. In mammals, AT-motif is present in many proteins, including high-mobility group A (HMGA) proteins, a family of non-histone chromosomal proteins, and hBRG1 protein, a central ATPase of the human switching/sucrose non-fermenting (SWI/SNF) remodeling complex. HMGA proteins act as architecture transcription factors to regulate many biological processes including growth, proliferation, differentiation and death, by binding to differently-spaced AT-rich DNA regions and/or interacting with several transcription factors.

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In plants, AT-hook family proteins have evolved in a unique way by harboring an AT-hook motif together with an uncharacterized Plant and Prokaryotes Conserved (PPC) domain. The PPC domain is also found in prokaryotic proteins, but they do not contain the AT-hook motif. The Arabidopsis genome contains a total of 29 AT-hook proteins (AHL1–29) and they have been shown to be involved in diverse processes, including hypocotyl elongation, flower development, gibberellin biosynthesis, leaf senescence, stem cell niche specification and root vascular tissue patterning. Among these, GIANT KILLER (GIK) is identified as a direct target of the floral homeotic protein AGAMOUS (AG), negatively fine-tuning multiple targets downstream of AG to control patterning and differentiation of reproductive organs through repressive histone modifications.

We thoroughly analyzed the other AT-hook members, and found TRANSPATABLE ELEMENT SILENCING VIA AT-HOOK (TEK) to be of particular interest, based on its high expression in the reproductive tissues, and the late flowering phenotype upon its knockdown.

Transposable elements (TEs) were discovered as “jumping genes” half a century ago by Barbara McClintock. Although they were primarily considered as parasites of host genome, recently a great amount of studies have uncovered the importance of TEs in genome function and evolution. TEs constitute a large fraction of most eukaryotic genomes including plants, e.g., 85% in maize and 17% in Arabidopsis. Activation of these “jumping genes” has a range of deleterious effects, including alterations of gene expression, gene deletions and insertions, and chromosome rearrangement. Epigenetic silencing helps to maintain genomic integrity by suppressing TE activities (reviewed in refs. 11 and 12). TEs are usually silenced by DNA methylation, repressive histone H3 lysine 9 dimethylation (H3K9me2), histone deacetylation and the presence of heterochromatic 14 nucleotides (nt) small interfering RNAs (siRNAs) that guide the RNA-directed DNA methylation (RdDM) machinery (reviewed in refs. 13 and 14). Recently, we have shown that the AT-hook DNA binding protein TEK is involved in the silencing of TEs and TE-like sequence containing genes, including LeF L C and FWA.

Figure 1. A model of TEK function. TEK binds to specific targets and makes a protein complex with FWA/MS5 and HDAC, which participates in histone deacetylation. Deacetylation of the target loci leads to transcriptional silencing. Once TEK action is abolished, the deacetylation process of its targets is blocked, resulting in the high acetylation level and reduced levels of both DNA methylation and H3K9 dimethylation at these loci, causing the transcriptional derepression of these targets.

The first noticeable phenotype in TEK knockdown plants is their extremely late flowering, which we later found that high expression of FWA and LeF L C is the main cause. The heritable and T-DNA-independent late flowering phenotypes in F1 of TEK knockdown line crossed with WT or T2 progeny from self-fertilized T1 knockdown lines suggest that the effects of TEK knockdown are very likely linked with the epigenetic control of FWA and LeF L C. Indeed, the LeF L C allele contains a 1224-bp insertion of a Mutator-like TE in the first intron, and FWA promoter contains SINE-related repeats, remnants of the TE, both of which are subjected to siRNA-mediated repression. In addition, ectopic expression of FWA in vegetative tissues is usually associated with loss of DNA methylation. However, bisulfite sequencing has only detected a slight reduction of DNA methylation in the CG, CHG and CHH contexts in the tandem repeats of FWA upon TEK knockdown. Microarray analysis further revealed that together with FWC and FWR, 1209 genes in total were upregulated at least 2-fold in TEK knockdown plants and among these, most (69%) are transposable element loci. AsMai is one of the TE genes upregulated in transgenic plants, and bisulfite sequencing also found that the percentages of methylated CG, CHG and CHH at AsMai locus were only modestly decreased. These data suggest that DNA methylation defect is neither the primary effect of TEK knockdown, nor the major cause of the upregulation of TE and TE-related genes. Instead, the levels of histone acetylation and H3K9me2 are dramatically changed upon TEK knockdown. Consistent with this observation, yeast-two-hybrid assay, bimolecular fluorescence complementation (BiFC) analysis and co-immunoprecipitation assay have shown that TEK protein interacts with Retinoblastoma-associated protein FVE and its homolog MS5, the components of histone deacetylation (HDAC) complexes. Thus, we proposed that TEK is involved in protecting genome stability partly by recruiting FVE/MS5-containing HDAC complexes to various target loci including FWA, FWR and TEs, which promotes a self-reinforcing cycle of histone deacetylation, DNA methylation and H3K9 dimethylation, leading to their transcriptional silencing. Upon TEK knockdown, the recruitment of histone deacetylation complex to the targets is abolished, resulting in the reduced levels of H3K9 dimethylation and DNA methylation, and the relatively higher levels of histone acetylation (summarized in Fig. I).
The transposition of at least two DNA transposons, 
CaCTa and hAt, were also observed in amiTEK plants by genic Southern blots.17 While the same epigenetic machinery could affect both transposition and transactivation of TEs, the study of transposition of TEs is limited (reviewed in 18). TE transposition is usually only found after inbreeding of mutants impaired in DNA methylation, in some cases after several generations.18 The immediate and dramatic burst of TE transposition was only detected at the combinational mutant of the loss of CG methylation and disruption of the siRNA pathway.19 The transposition of TEs in amiTEK T1 lines, with the observation of the slight reduction of CG methylation, indicates the possibility that TEK knockdown affects the siRNA pathway in Arabidopsis.

The characterized function of Arabidopsis TEK is quite comparable to another well-studied AT-hook protein SATB1 in mammals. SATB1 acts as a global repressor of cell function in specific cell lineages. The binding of SATB1 affects the chromatin status of target loci and modifies the histone acetylation status.20 In Arabidopsis, SATB1 was also observed in the flower and is involved in transposon silencing.21 The transposition of at least two interacting AT-hook factors in Arabidopsis reveals that TEK knockdown affects the siRNA pathway in Arabidopsis.16

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