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Plant homologs of mammalian MBT-domain protein-regulated KDM1 histone lysine demethylases do not interact with plant Tudor/PWWP/MBT-domain proteins

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ABSTRACT

Histone lysine demethylases of the LSD1/KDM1 family play important roles in epigenetic regulation of eukaryotic chromatin, and they are conserved between plants and animals. Mammalian LSD1 is thought to be targeted to its substrates, i.e., methylated histones, by an MBT-domain protein SFMBT1 that represents a component of the LSD1-based repressor complex and binds methylated histones. Because MBT-domain proteins are conserved between different organisms, from animals to plants, we examined whether the KDM1-type histone lysine demethylases KDM1C and FLD of Arabidopsis interact with the Arabidopsis Tudor/PWWP/MBT-domain SFMBT1-like proteins SL1, SL2, SL3, and SL4. No such interaction was detected using the bimolecular fluorescence complementation assay in living plant cells. Thus, plants most likely direct their KDM1 chromatin-modifying enzymes to methylated histones of the target chromatin by a mechanism different from that employed by the mammalian cells.

1. Introduction

Post-translational histone modifications, e.g., acetylation, methylation, and ubiquitination, play central role in gene regulation in all eukaryotic organisms, determining the active or inactive state of the chromatin. These modifications are effected by diverse histone-modifying enzymes, such as histone deacetylases, histone lysine demethylases, histone methyltransferases, and histone deubiquitinases. Among those, LSD1/KDM1-type histone lysine demethylases [1] represent one of the most recently discovered [2], yet clearly central factors in controlling chromatin in different organisms, from animals to plants [3–5]. In animal calls, LSD1/KDM1A promotes demethylation of dimethylated lysine 4 (K4) of histone H3 [2] and often functions as negative regulator of gene expression [6]. LSD1/KDM1A usually acts in complex with CoREST that modulates the gene repression function of LSD1 *in vivo*, a histone methyltransferase (HMT; e.g., G9a), HDAC1/2 histone deacetylases, and DNA binding zinc finger proteins (e.g., either REST or ZNF217) [2,7–17]. However, none of these components recognize methylated histones that represent the direct substrates of LSD1/KDM1A; recently, this role has been attributed to the SFMBT1 [Scm (Sex comb on midleg) with four MBT (malignant brain tumor) domains 1] protein, which functions as part of the LSD1/KDM1A-based repressor complex and is known to bind different forms of methylated histones [18,19].

In plants, our knowledge of LSD1/KDM1 complexes is just emerging. For example, Arabidopsis, one of the major model plants, encodes four KDM1 proteins, KDM1A (FLD), KDM1B, KDM1C, and KDM1D [1]. KDM1C has been shown to interact with histone methyltransferase SURV5 [20–22] and histone deubiquitinase OTLD1 [23]. MBT domains are conserved within proteins from different organisms, from animals to insects to plants [24,25]. Thus, we examined whether Arabidopsis LSD1/KDM1A-type histone lysine demethylases can recognize plant Tudor/PWWP/MBT-domain proteins [24,25], i.e., the Arabidopsis SFMBT1-like proteins (SLs). Our data indicate no such interactions and suggest that plant LSD1/KDM1 histone lysine demethylases are directed to their substrates by a mechanism different from their mammalian counterparts.

2. Materials and methods

2.1. Plasmid construction

The coding sequences of *SL1*, *SL2*, *SL3*, and *SL4*, *KDM1C* and *FLD*

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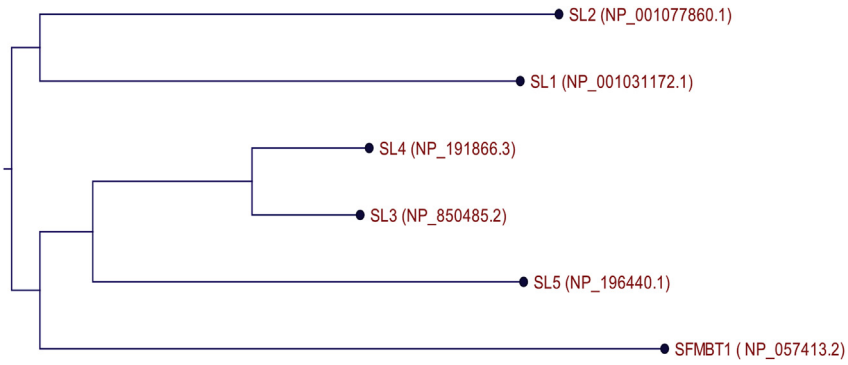

Fig. 1. Phylogenetic tree of human SFMBT1 and its Arabidopsis SL1, SL2, SL3, SL4, and SL5 homologs. The SL proteins were identified by the BLASTA search [33]. GenBank accession numbers are shown in parentheses next to each protein name. The tree was built by the CLC Main Workbench 7.6.4 software (http://www.clcbio.com), using default parameters.

were amplified from *Arabidopsis thaliana* cDNA library using primers detailed in Table S1. The *SL5* sequence failed to amplify and was not examined in this work. For transient expression in *Nicotiana benthamiana*, the amplified *SL1*, *SL2*, *SL3*, and *SL4* as well as *SURV5* [20] were cloned into the HindII-SalI, EcoRI-SalI, XhoI-SalI, SalI-SacII, and SalI sites of pSAT4-nEYFP [26], respectively, resulting in pSAT4-SL1-nYFP, pSAT4-SL2-nYFP, pSAT4-SL3-nYFP, pSAT4-SL4-nYFP, and pSAT4-SURV5-nYFP. The amplified *KDM1C* and *KDM1A* (*FLD*) were cloned into the XhoI-SmaI and SacI-SalI sites of pSAT1-cEYFP [26], respectively, resulting in pSAT1-cYFP-KDM1C and pSAT1-cYFP-FLD. Then, the expression cassettes from pSAT1-cYFP-KDM1C and pSAT1-cYFP-FLD were transferred into the AscI site of the binary vector for multigene expression pPZP-RCSII [27], resulting in pRCSII-KDM1C and pRCSII-FLD. Finally, the expression cassettes from pSAT4-SL1-nYFP, pSAT4-SL2-nYFP, pSAT4-SL3-nYFP, and pSAT4-SL4-nYFP were transferred into the I-SceI site of pRCSII-KDM1C and pRCSII-FLD, resulting in pRCSII-KDM1C-SL1, pRCSII-KDM1C-SL2, pRCSII-KDM1C-SL3, pRCSII-KDM1C-SL4, pRCSII-FLD-SL1, pRCSII-FLD-SL2, pRCSII-FLD-SL3, and pRCSII-FLD-SL4. For positive control experiments, the expression cassette from pSAT4-SURV5-nYFP was subcloned into the I-SceI site of pRCSII-KDM1C, resulting in pRCSII-KDM1C-SURV5.

2.2. BiFC assay

N. benthamiana plants were grown to a six-leaf stage at 25 °C with 16 h light (70–80 μmol photons m^{-2} s^{-1}) and 8 h dark. The tested proteins were transiently expressed in *N. benthamiana* plants by agroinfiltration as described [28] with modifications. Briefly, the binary constructs were transferred into *Agrobacterium tumefaciens* strain EHA105 by heat shock. The bacterial cells then were grown on LB medium supplemented with rifampicin (25 μg mL^{-1}) and spectinomycin (100 μg mL^{-1}) at 28 °C for 48 h, resuspended in the infiltration buffer (10 mM $MgCl_2$, 10 mM MES pH 5.6, 150 mM acetsosyringone) to $A_{600} = 1.0$, and grown at room temperature for another 2–3 h. The abaxial side of mature leaves of 4–5-week-old *N. benthamiana* plants was vacuum-infiltrated with bacterial cell suspension harboring each tested construct. Plants were grown for additional 72 h, and the BiFC signal in the infiltrated areas was detected using a Zeiss LSM 5 Pa confocal microscope. All experiments were repeated at least three times.

3. Results and discussion

3.1. Arabidopsis SL proteins

Amino acid sequence analysis identified five relatively close homologs of the human SFMBT1 protein encoded by the Arabidopsis genome. These sequences were aligned with SFMBT1 by CLC Main Workbench 7.6.4 (http://www.clcbio.com) software, using default parameters (Fig. S1), and their phylogenetic tree was generated (Fig. 1). All of these five genes, i.e., *SL1* (At1g51745), *SL2* (At1g80810), *SL3* (At2g48160), *SL4* (At3g63070), and *SL5* (At5g08230) belong to the Tudor domain "Royal family", which includes proteins with Tudor, plant Agenet, Chromo, PWWP and MBT domains [24]. Protein products of the *SL3*, *SL4*, and *SL5* genes, known as members of the *HULK* gene family, localize in the plant cell nucleus and play important roles in regulating flowering timing [29]. The presence of Tudor/PWWP/MBT domains raised a possibility that these plant proteins, similarly to their mammalian homologs [18,19], might act to direct KDM1 proteins to their sites of action on the target chromatin. In this scenario, the SL proteins should interact with KDM1 proteins. This idea was examined directly by monitoring potential KDM1-SL interactions in the living plant cells.

3.2. KDM1A and KDM1C do not recognize the SL1-4 proteins

Mammalian SFMBT1 is suggested to interact with LSD1/KDM1A through another component of the repressor complex CoREST [18]; however, plants do not encode CoREST homologs, suggesting that such interactions may happen directly, without the involvement of CoREST-like proteins. Thus, we assayed whether the SL1, SL2, or SL3 and SL4 proteins can interact with two best-studied representatives of the Arabidopsis KDM1 family, KDM1A (FLD) and KDM1C [1,20,30,31]. The potential interactions were tested using bimolecular fluorescence complementation (BiFC) within living plant cells [26,32]. Each of the tested KDM1 and SL proteins were tagged with carboxyl- and amino-terminal fragments of YFP, respectively, and transiently coexpressed in *N. benthamiana* leaves.

Fig. 2 shows that coexpression of cYFP-FLD with nYFP-SL1, nYFP-SL2, nYFP-SL3, or nYFP-SL4 did not produce a BiFC signal, indicating that FLD was unable to interact with any of these SL1-4 proteins. In positive control experiments, the YFP fluorescence was reconstituted following coexpression of two known interactors KDM1C and SURV5 [20] tagged with cYFP and nYFP, respectively. As expected [20], the cYFP-KDM1C-nYFP-SURV5 complexes were observed in the cell nuclei (Fig. 2).

Coexpression of cYFP-KDM1C with nYFP-SL1, nYFP-SL2, nYFP-SL3, or nYFP-SL4 failed to regenerate the YFP signal, indicating no interaction between KDM1C and any of these SL proteins (Fig. 3). In this set of experiments, the positive control also detected the KDM1C-SURV5 interaction following coexpression of cYFP-KDM1C and nYFP-SURV5, with the interacting proteins accumulating in the