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Research Article

SLIDE, the Protein Interacting Domain of Imitation Switch Remodelers, Binds DDT-Domain Proteins of Different Subfamilies in Chromatin Remodeling Complexes[F]

Jiaqiang Dong[1†], Zheng Gao[1,2†], Shujing Liu[1†], Guang Li[1††], Zhongnan Yang[2], Hai Huang[1] and Lin Xu[1*]

[1]National Laboratory of Plant Molecular Genetics, Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences, Shanghai 200032, China

[2]College of Life and Environment Sciences, Shanghai Normal University, Shanghai 200234, China

[†]These authors contributed equally to this work.

[††]Current address: Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA, 94305, USA

[*]Corresponding author

Tel: +86 21 5492 4085; Fax: +86 21 5492 4015; E-mail: xulin01@sibs.ac.cn

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Abstract

The Imitation Switch (ISWI) type adenosine triphosphate (ATP)-dependent chromatin remodeling factors are conserved proteins in eukaryotes, and some of them are known to form stable remodeling complexes with members from a family of proteins, termed DDT-domain proteins. Although it is well documented that ISWIs play important roles in different biological processes in many eukaryotic species, the molecular basis for protein interactions in ISWI complexes has not been fully addressed. Here, we report the identification of interaction domains for both ISWI and DDT-domain proteins. By analyzing CHROMATIN REMODELING11 (CHR11) and RINGLET1 (RLT1), an *Arabidopsis thaliana* ISWI (AtISWI) and AtDDT-domain protein, respectively, we show that the SLIDE domain of CHR11 and the DDT domain together with an adjacent sequence of RLT1 are responsible for their binding. The *Arabidopsis* genome contains at least 12 genes that encode DDT-domain proteins, which could be grouped into five subfamilies based on the sequence similarity. The SLIDE domain of AtISWI is able to bind members from different AtDDT subfamilies. Moreover, a human ISWI protein SNF2H is capable of binding AtDDT-domain proteins through its SLIDE domain, suggesting that binding to DDT-domain proteins is a conserved biochemical function for the SLIDE domain of ISWIs in eukaryotes.

Keywords: *Arabidopsis*; chromatin remodeling factors; DDT-domain proteins; Imitation Switch; protein interaction.

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Introduction

Adenosine triphosphate (ATP)-dependent chromatin remodeling factors (remodelers) play critical roles in gene regulation by dynamically packaging or unpackaging chromatin using energy from ATP hydrolysis (Clapier and Cairns 2009). By forming remodeling complexes with other proteins, remodelers change contacts between histones and DNA in the nucleosome, thereby facilitating or blocking the accessibility of DNA-binding proteins to specific DNA sequences (Clapier and Cairns 2009). The *Arabidopsis thaliana* genome contains 41 genes that encode such ATPase remodelers, some of which have been extensively characterized and shown to act as important developmental regulators (Knizewski et al. 2008).

The Imitation Switch (ISWI) type chromatin remodeling factors belong to a subfamily of these remodelers. In *A. thaliana* there are two ISWI (AtISWI) proteins: CHROMATIN REMODELING 11 (CHR11) and CHR17 (Knizewski et al. 2008). Through experiments involving RNA interference, *CHR11* was shown to be required for female gametogenesis (Huanca-Mamani et al. 2005). More recent studies by characterization of the *chr11 chr17* double mutant revealed that CHR11 and CHR17 act redundantly to maintain the plant vegetative phase by regulating several key genes involved in plant reproductive development (Li et al. 2012a). AtISWI proteins were also found to be associated with protein complexes of the MADS-box transcription factors in flowers, suggesting a role for AtISWI in flower development (Smaczniak et al. 2012).

It was reported that ISWI remodelers usually act in a protein complex in different species, and ISWI complexes frequently contain proteins with a structurally conserved domain, called the DDT domain (Doerks et al. 2001; Yadon and Tsukiyama 2011). In *Drosophila melanogaster*, two DDT-domain proteins, ACF1 and NURF301, are the important components in ISWI complexes. Either of these two proteins can be presented in three distinct *Drosophila* ISWI (dISWI) complexes, namely ACF (Ito et al. 1997, 1999), CHRAC (Varga-Weisz et al. 1997; Eberharter et al. 2001), or NURF (Tsukiyama and Wu 1995; Tsukiyama et al. 1995; Xiao et al. 2001). In yeast, the ISWI protein Isw2 forms a complex with a DDT-domain protein, Itc1 (Gelbart et al. 2001; Sugiyama and Nikawa 2001). In humans, DDT-domain proteins TIP5 and WSTF have also been found in ISWI complexes (Lu et al. 1998; Strohner et al. 2001; Bozhenok et al. 2002). Maintenance of the plant vegetative phase by AtISWI proteins CHR11 and CHR17 also requires the AtDDT-domain proteins. Not only do CHR11 and CHR17 bind directly to the AtDDT-domain proteins RINGLET1 (RLT1) and RLT2, but phenotypes of the *rlt1 rlt2* double mutant resemble those of *chr11 chr17* (Li et al. 2012a).

Although ISWI complexes containing the DDT-domain proteins have been reported from different eukaryotic species, the molecular basis for interaction of these two types of proteins is not fully addressed. In this study, we report the identification of binding domains for both AtISWI and AtDDT-domain proteins. We propose that binding between the SLIDE domain of ISWI and diverse DDT-domain proteins is a conserved molecular basis for the ISWI complex in eukaryotic kingdoms.

Results

chr11 chr17 displays more severe developmental defects than those of *rlt1 rlt2*

Previous data showed that both *chr11-1 chr17-1* and *rlt1-1 rlt2-1* double mutants have some similar developmental defects, including reduced plants size, earlier transition from vegetative to reproductive growth, and homeotic transformation from leaf to floral organ tissues (Li et al. 2012a). These similar plant phenotypes suggest that CHR11/17 and RLT1/2 proteins at least partially act in the same genetic pathways, and this idea was further supported by the direct interaction between the CHR11/17 and RLT1/2 proteins (Li et al. 2012a). However, compared with *rlt1-1 rlt2-1*, the *chr11-1 chr17-1* double mutants exhibited more severe developmental defects. For example, although both *chr11-1 chr17-1* and *rlt1-1 rlt2-1* showed the earlier flowering time compared to the wild-type Columbia-0 (Col-0), *chr11-1 chr17-1* flowered even earlier (Li et al. 2012a). In addition, *chr11-1 chr17-1* roots were also drastically shortened compared with those of *rlt1-1 rlt2-1* and the wild type (**Figure 1A, B**). Scanning electron microscopy (SEM) showed that trichomes of the first-pair rosette leaves usually have two to four branches in the wild-type (**Figure 1C, F**) and *rlt1-1 rlt2-1* (**Figure 1E, F**) plants, whereas they have five or six branches in the *chr11-1 chr17-1* plants (**Figure 1D, F**). These observations suggest that other partially redundant AtISWI remodeling complexes may exist, which might contain the AtDDT-domain proteins other than RLTs. To elucidate this possibility, understanding of the molecular basis for AtISWI and RLT interactions and identifying new factors that interact with AtISWI are important.

SLIDE domain of ISWI is responsible for protein interaction with RLTs

The ISWI-type remodelers are widely present in eukaryotes and are structurally highly conserved especially in the ATPase, HAND, SANT and SLIDE domain sequences (Clapier and Cairns 2009; Yadon and Tsukiyama 2011). CHR11 has a typical ISWI structure with the ATPase domain at the N-terminus and the HAND, SANT and SLIDE domains at the C terminus (**Figure 2A**). Between SANT and SLIDE, there is an intermediate region, termed Spacer, which is composed of an α helix (Grune et al. 2003; **Figure 2A**). To investigate the interaction between AtISWI and AtDDT-domain proteins, we first truncated the CHR11 proteins into N- and C-terminal parts and tested their binding activities with a full-length AtDDT-domain protein, RLT1, using a yeast two-hybrid assay (**Figure 2A**). While the N-terminal region of CHR11 did not interact with RLT1, the C-terminal region bound strongly to RLT1 in yeast cells (**Figure 2A**). We further truncated the CHR11 C-terminal region by removing HAND, SANT, half of Spacer and the entire region downstream to the SLIDE domain, but the remaining portions all bound to RLT1 (**Figure 2A**). However, the truncated portion containing an incomplete SLIDE domain lost the ability to bind to RLT1 (**Figure 2A**), indicating that the SLIDE domain is responsible for the interaction with DDT-domain proteins.

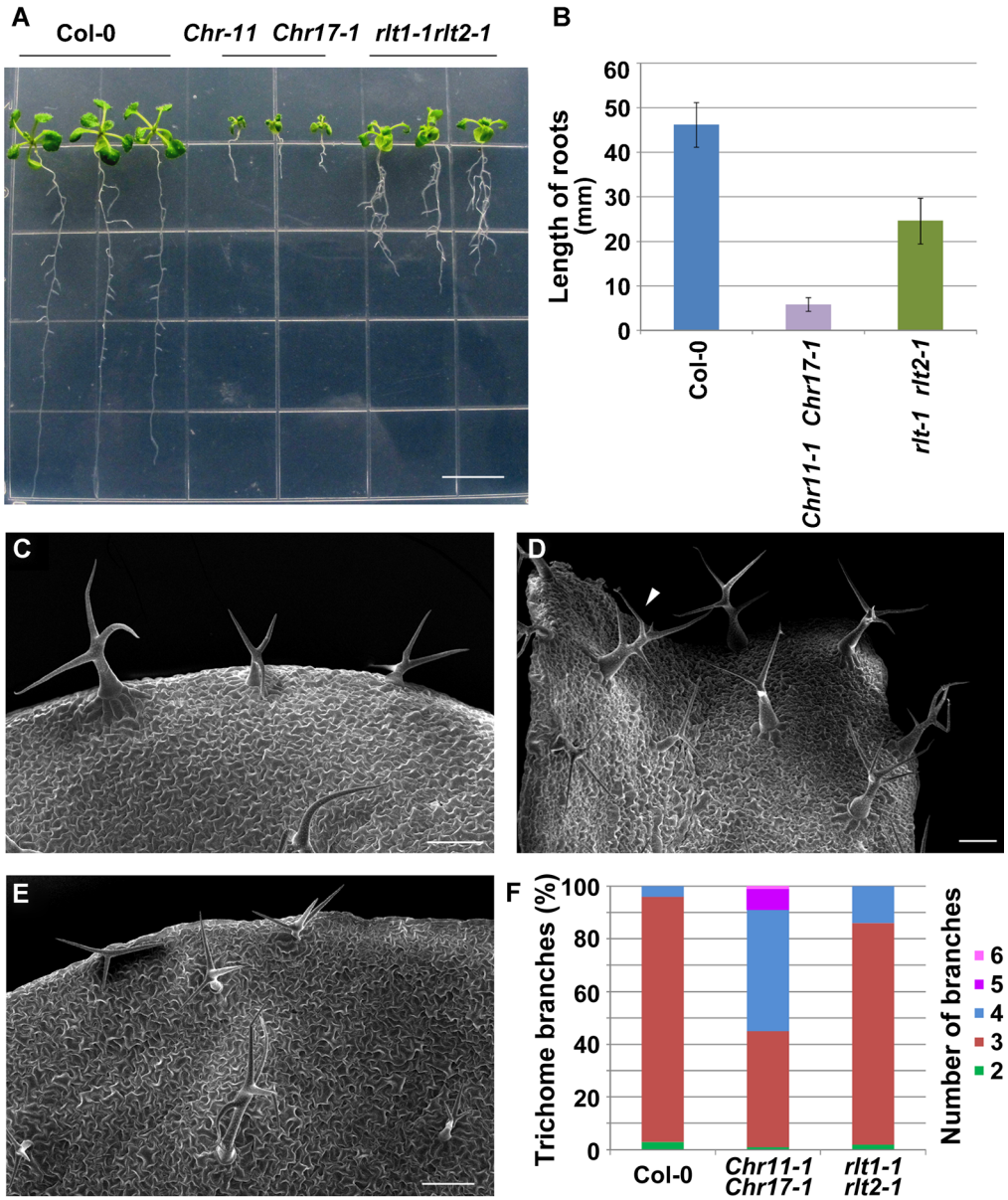

Figure 1. ***chr11-1 chr17-1*** **demonstrates more strong phenotypic abnormalities than** ***rlt1-1 rlt2-1.***

(A) Root phenotypes of 14-d-old seedlings of wild-type (left), *Chr11-1 Chr17-1* (middle) and *rlt1-1 rlt2-1* (right).
(B) Statistic analysis of the root length of 14-d-old seedlings. $n = 20$. Bars show *SE*.
(C–E) Scanning electron microscopy (SEM) analysis of trichome branches on the adaxial side of the first-pair rosette leaves of wild type (**C**), *Chr11-1 Chr17-1* (**D**), and *rlt1-1 rlt2-1* (**E**). An arrowhead in (**D**) indicates a trichome with five branches in *Chr11-1 Chr17-1*.
(F) Statistic analysis of trichome branches. $n = 100$.
Bars = 10 mm in (**A**) and 100 μm in (**C–E**).

We next carried out a truncation analysis to determine the protein interaction domain for RLT1, and the truncated RLT1 portions were tested for their interactions with SLIDE of CHR11 using the yeast two-hybrid assay. The interaction domain of RLT1 was localized to a region containing the DDT domain together with the D-TOX B, C and D motifs (**Figure 2B**). Hence, although the DDT domain is the most conserved part among the DDT-domain proteins of different species, this domain is necessary but may not be sufficient for interactions between the AtISWI and AtDDT-domain proteins.

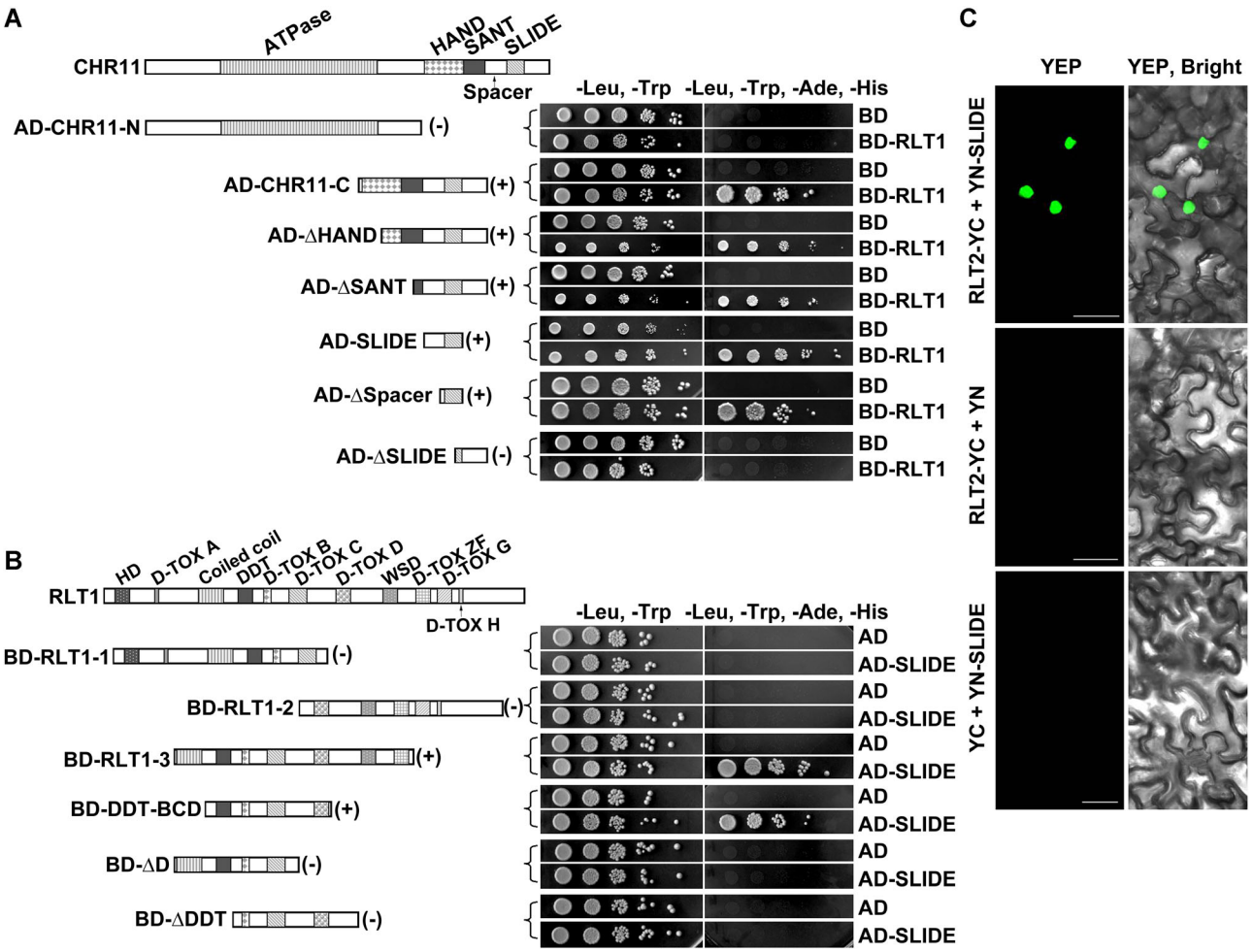

Figure 2. Truncation analyses to identify domains required for the protein interaction of CHR11 and RLT1.

(A and **B)** Truncation analyses using the yeast two-hybrid assay to identify the protein interaction domains for CHR11 (**A**) and RLT1 (**B**). (+) and (−) indicate positive and negative protein interactions in yeast cells, respectively. Distributions of domains or motifs are marked above the full-length proteins according to previous reports (Mukherjee et al. 2009; Li et al. 2012a).

(C) The BiFC assay shows the in planta interaction between the SLIDE domain of CHR11 and the RLT2 full-length protein in tobacco leaves. Note that both negative controls of RLT2-YC + YN and YC + YN-SLIDE showed no interaction signals.

Bars = 50 μm in (**C**).

RLT1 and RLT2, both of which belong to the class-I subfamily of AtDDT-domain proteins (see below), share a high overall sequence similarity and act redundantly in maintaining plant vegetative development (Li et al. 2012a). To test the in planta interaction between the SLIDE domain of CHR11 and RLT proteins, we performed a bimolecular fluorescence complementation (BiFC) assay using the RLT2 protein (**Figure 2C**). Tobacco leaves carrying the *35S$_{pro}$:YN-SLIDE* and *RLT2$_{pro}$:RLT2-YC* pair exhibited YFP fluorescence, while those carrying the *35S$_{pro}$:YN* and *RLT2$_{pro}$:RLT2-YC* or the *35S$_{pro}$:YN-SLIDE* and *RLT2$_{pro}$:YC* pair did not. These data support the notion that the SLIDE domain of AtISWI proteins is responsible for the AtDDT-AtISWI interaction in plants.

Interactions between AtISWI and other AtDDT-domain proteins

To gain more information about the AtDDT-domain proteins, we analyzed the whole *Arabidopsis* genome and identified a total of 12 genes that encode proteins with the DDT domain, constituting the AtDDT family. Based on sequence similarity and domain distribution, these AtDDT-domain proteins were classified into five subfamilies (class I through V) (**Figure 3A, B**). Members in

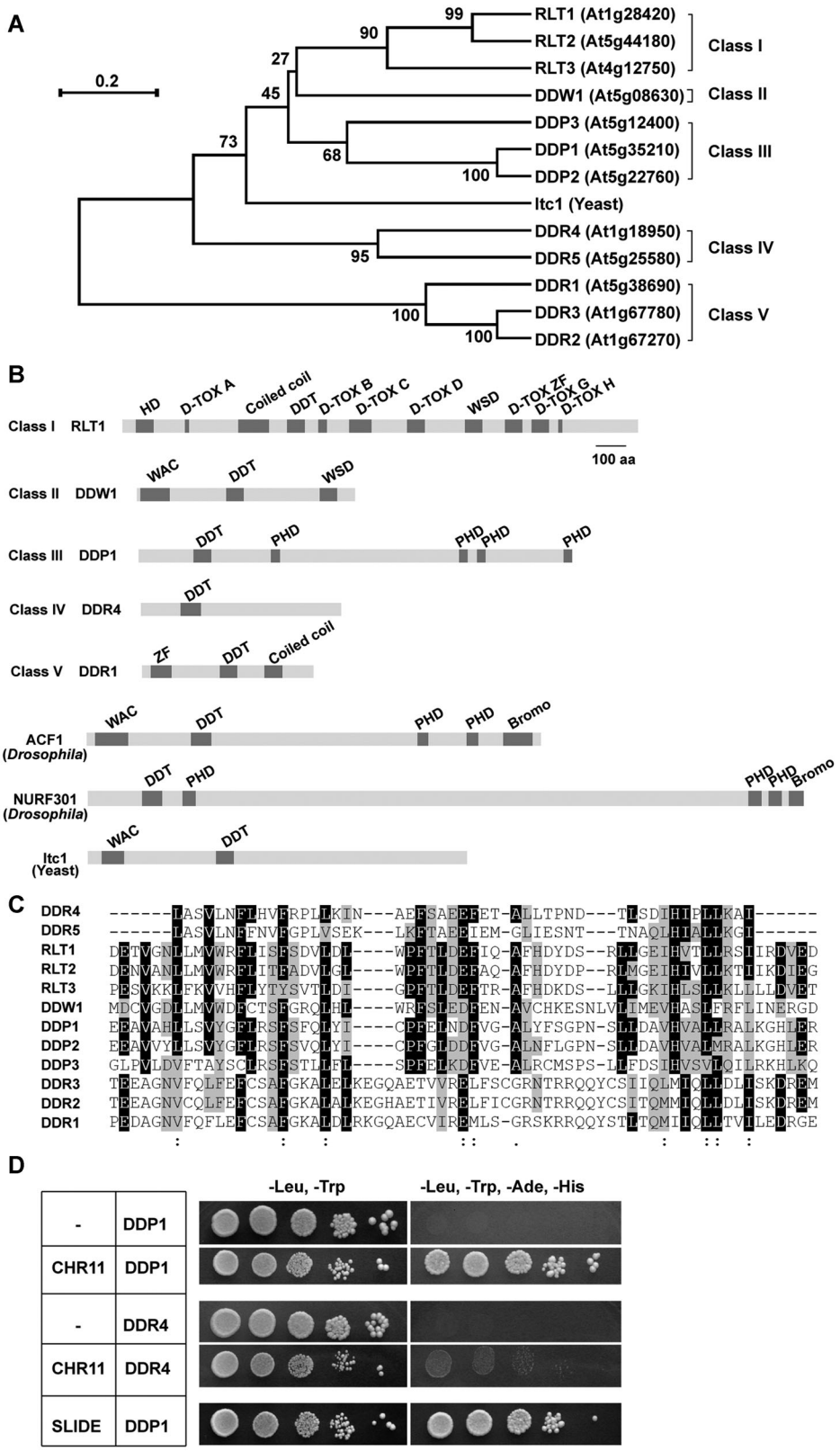

Figure 3. Continued.

each subfamily share a higher sequence similarity and also possess similar domains except for class II, which contains only one member. The class-I proteins are plant specific, including RLT1, RLT2 and RLT3. In addition to the conserved DDT domain, the class-I proteins also contain a homeodomain (HD), several D-TOX motifs, and a WSD domain (Mukherjee et al. 2009) (**Figure 3B**). The class-II protein DDT-WAC PROTEIN1 (DDW1) contains an additional WAC domain in addition to the DDT and WSD domains (**Figure 3B**). The structure of this class-II protein is similar to that of a yeast DDT-domain protein Itc1 (**Figure 3B**; Doerks et al. 2001). The class-III members include DDT-PHD PROTEIN1 (DDP1), DDP2 and DDP3, which all carry multiple PHD domains (**Figure 3B**). These class-III proteins are structurally similar to the *Drosophila* DDT-domain proteins ACF1 and NURF301 but lacking a bromodomain (Doerks et al. 2001) (**Figure 3B**). The class-IV and -V AtDDT-domain proteins are also plant specific and the members include DDT-RELATED PROTEIN1 (DDR1) through DDR5 (**Figure 3B**). Like those in other eukaryotic species, the relatively conserved domain in all these AtDDT-domain proteins is the DDT domain (**Figure 3C**).

We selectively analyzed protein interactions of the class-III member DDP1 and the class-IV member DDR4 with either the full-length CHR11 protein or the SLIDE domain of CHR11, using the yeast two-hybrid assay. Our data showed that the DDP1 protein strongly bound to CHR11 in yeast cells, while a very weak interaction between DDR4 and CHR11 was also detected (**Figure 3D**). Similarly, the SLIDE domain of CHR11 was found to interact with DDP1 (**Figure 3D**). To further confirm the protein interaction between CHR11 and the class-III DDT-domain proteins, we performed a BiFC assay to test the in planta interaction using CHR11 and DDP2. Tobacco leaf epidermal cells carrying the *35S$_{pro}$:YN-CHR11* and *35S$_{pro}$:DDP2-YC* pair showed clear YFP fluorescence in the nucleus, while those harboring the *35S$_{pro}$:YN* and *35S$_{pro}$:DDP2-YC* and the *35S$_{pro}$:YN-CHR11* and *35S$_{pro}$:YC* pairs produced no fluorescence (Figure S1). These data suggest that class-III and -IV proteins are able to bind AtISWI. We also analyzed protein interactions between CHR11 and the class-II and -V AtDDT-domain proteins, DDW1 and DDR1, respectively, using yeast two-hybrid, but failed to obtain the positive results. Previous study using liquid chromatography-tandem mass spectrometry (LC-MS/MS) showed that the AtISWI complexes may contain different types of AtDDT family proteins, including RLT1, RLT2, DDP2, DDP3, DDR1, and DDW1 (Smaczniak et al. 2012). It is possible that AtISWI is able to bind to AtDDT-domain proteins of all subfamilies through their SLIDE domains, and the negative CHR11-DDW1 and -DDR1 interactions in our yeast two-hybrid analyses may result from lacking of correct higher structures for the engineered proteins or assistant proteins for the interaction in the yeast cells.

DDT-SLIDE interaction is evolutionarily conserved

The SLIDE domain is structurally conserved in eukaryotes (**Figure 4A**) and is composed of three helices (**Figure 4B**; Grune et al. 2003). To test whether protein binding for the SLIDE domain is conserved, we examined a potential interaction between the human ISWI protein SNF2H and RLT1 or DDP1 using the yeast two-hybrid assay. Our results showed that SNF2H strongly bound to RLT1 and DDP1 in yeast cells, and again, the interaction required the SLIDE domain of SNF2H (**Figure 4C**). These results suggest that the molecular basis for the interaction between the SLIDE domain and DDT-domain proteins is evolutionarily conserved.

Discussion

Protein interactions between ISWI remodelers and DDT-domain proteins were found from a variety of eukaryotic species. In addition to those in yeast, animals, and human, direct protein interactions between AtISWI and AtDDT-domain proteins have also been reported in plants. For example, an LC-MS/MS analysis showed that several different AtDDT-domain proteins are present in the AtISWI complexes (Smaczniak et al. 2012), and the *Arabidopsis* proteins CHR11 and CHR17 are able to form complexes with RLT1 and RLT2 (Li et al. 2012a). In this study, we further demonstrated that CHR11 can bind to the class-III and -IV AtDDT-domain proteins DDP1 and DDR4, respectively, albeit a weak binding of CHR11 and DDR4 in yeast cells. Thus, a functional ISWI complex containing ISWI and

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Figure 3. Bioinformatic analysis of AtDDT-domain proteins.

(A) Cladogram of the AtDDT family proteins. AtDDT-domain proteins were identified from the TAIR database (http://www.arabidopsis.org). These proteins could be further classified into five subfamilies according to the specific domain types and the domain distribution. The DDT domain sequences were used in the phylogenic analysis, using the MEGA3.0 package (Kumar et al. 2004).

(B) Schematic diagram of domain distribution of AtDDT-domain proteins. ACF1 and NURF301 are two typical DDT-domain proteins from *Drosophila*, and Itc1 is the yeast DDT-domain protein.

(C) Alignment of the DDT domain of the AtDDT-domain proteins, using the ClustalX2 (Larkin et al. 2007) and BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html) packages.

(D) DDP1 and DDR4 proteins interact with CHR11 in a yeast two-hybrid assay.

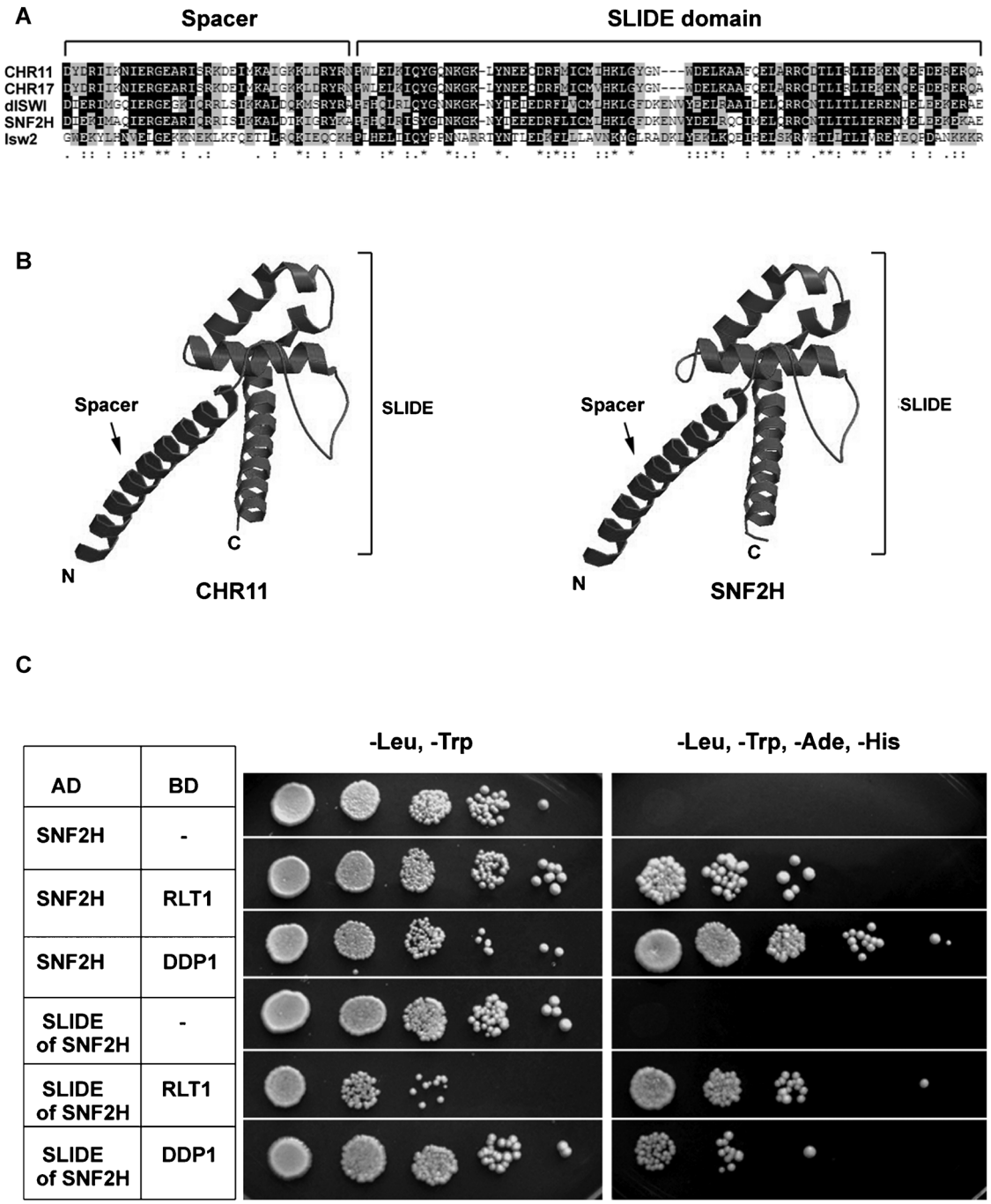

Figure 4. The human Imitation Switch (ISWI) protein SNF2H has the ability to bind AtDDT-domain proteins.

(A) Alignment of the Spacer region and the SLIDE domain in several selected eukaryotic species, using the ClustalX2 (Larkin et al. 2007) and BioEdit packages (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

(B) Predicted 3D structures of the Spacer region and the SLIDE domain of CHR11 (left) and SNF2H (right) using the SWISS-MODEL program (Arnold et al. 2006) (http://swissmodel.expasy.org/). The structures of these two proteins are similar.

(C) The yeast two-hybrid assay shows that full-length SNF2H and the SLIDE domain of SNF2H interact with RLT1 and DDP1.

DDT-domain proteins for chromatin remodeling is likely common in eukaryotes.

Previous findings and the data in this study all indicate that AtISWI remodelers play roles in multiple developmental processes (Huanca-Mamani et al. 2005; Li et al. 2012a; Smaczniak et al. 2012). However, *Arabidopsis* has only two ISWI members, CHR11 and CHR17, and functions of these two AtISWI proteins are highly redundant as plants with single mutation for any of these AtISWI genes are normal (Li et al. 2012a). One possibility for AtISWI to have so many different functions in plant development is that they may form complexes with different AtDDT-domain proteins. Because varieties of the AtDDT-domain protein are much more than those of AtISWIs, there are likely more types of AtISWI complexes with different physiological functions than those of the AtISWI types. As different AtISWI complexes may play distinct roles in regulation of chromatin structure, this diversity may lead to varying biochemical functions of AtISWI in epigenetic gene regulations. For example, the DDP proteins possess the PHD domain, which was proposed to bind methylated histones (Wysocka et al. 2006), and RLT proteins contain the homeodomian, which may have a function to bind DNA (Mukherjee et al. 2009). Thus, both DDP-AtISWI and RLT-AtISWI complexes may be located at specific positions either by binding methylated histones or DNA sequences. Epigenetic regulations are important for plant development and physiology (Postnikova et al. 2011; Di Giacomo et al. 2013). It would be of interest in the future to analyze individual AtISWI complexes and to determine their specific physiological and biochemical functions in planta.

Our study revealed that the SLIDE domain of ISWIs is responsible for the interaction with DDT-domain proteins (**Figure 5**). This protein interaction is probably highly conserved in different eukaryotic species, as we show in this study that even the SLIDE domain from the human ISWI protein can strongly bind AtDDT-domain proteins. Since the protein structure of ISWIs is conserved in eukaryotes (Grune et al. 2003; Dang and Bartholomew 2007; Yadon and Tsukiyama 2011; Yamada et al. 2011; Li et al. 2012a), the basic biochemical function for ISWI proteins may be conserved. DDT is the only domain that is structurally conserved for all DDT-domain proteins. It is thus possible that the DDT domain and its neighboring sequences are essential for the direct interaction with ISWIs, whereas specific DDT-ISWI functions may rely on the distinct parts of the DDT-domain proteins.

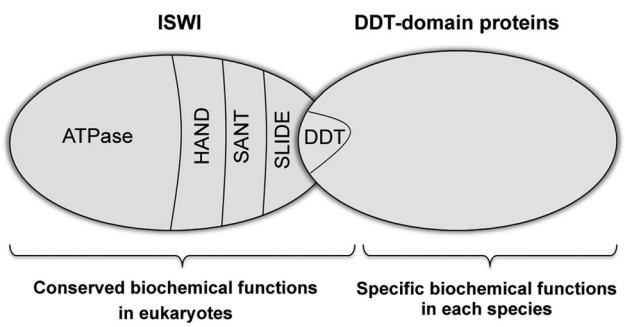

Figure 5. A model for the interaction of DDT-domain and Imitation Switch (ISWI) proteins.

According to the data in this study, the SLIDE domain binds to the DDT domain together with certain DDT neighboring sequences, which might play roles for the stability of the DDT domain.

Materials and Methods

Plant materials and SEM

Plant (*Arabidopsis thaliana* L.) mutants of *chr11-1 chr17-1* and *rlt1-1 rlt2-1* are in the Col-0 background and were previously described (Li et al. 2012a). SEM was performed according to our previous method (Xu et al. 2003).

Yeast two-hybrid analysis

The full length *DDP1*, *DDR4* and *SNF2H* cDNAs and the cDNAs encoding the truncated CHR11, RLT1, or SNF2H proteins were subcloned into pGADT7 and pGBKT7 vectors (Clontech, Mountain View, CA, USA), respectively. *pGADT7-CHR11* and *pGBKT7-RLT1* were described previously (Li et al. 2012a). Primers used in plasmid construction were listed in Table S1. The yeast two-hybrid assay was performed according to a protocol recommended by the manufacturer (Clontech).

BiFC

For the BiFC assay, the cDNA of the SLIDE domain of CHR11 with a 5′ fusion of 3× FLAG or the genomic DNA encoding the DDP2 protein was subcloned into the plant transformation vector pCAMBIA1300-35S (modified from pCAMBIA1300, Cambia, Canberra, Australia) under the control of the 35S promoter to result in $35S_{pro}$*:3× FLAG-SLIDE* or $35S_{pro}$*:DDP2*. The $35S_{pro}$*:YN-SLIDE* or $35S_{pro}$*:DDP2-YC* fusion was constructed by insertion of the cDNA encoding the YN or YC fragment (N or C terminus of truncated YFP at residue 155) into the 5′ end of the $35S_{pro}$*:3× FLAG-SLIDE* plasmid to replace *3× FLAG* or 3′ end of the $35S_{pro}$*:DDP2* plasmid, respectively. Sequences between YN and SLIDE or DDP2 and YC contain the GGGS linker peptide together with the FLAG tag (Kerppola 2006). Primers used in plasmid construction are listed in Table S1. Construction of the

RLT2$_{pro}$:RLT2-YC, *RLT2$_{pro}$:YC*, *35S$_{pro}$:YN-CHR11*, *35S$_{pro}$:YN* and *35S$_{pro}$:YC* plasmids and the BiFC method were described previously (Li et al. 2012a, 2012b).

Accession numbers

The *Arabidopsis* Genome Initiative locus identifiers are *CHR11* (At3g06400), *CHR17* (At5g18620), *RLT1* (At1g28420), *RLT2* (At5g44180), *RLT3* (At4g12750), *DDW1* (At5g08630), *DDP1* (At5g35210), *DDP2* (At5g22760), *DDP3* (At5g12400), *DDR1* (At5g38690), *DDR2* (At1g67270), *DDR3* (At1g67780), *DDR4* (At1g18950), and *DDR5* (At5g25580). The GenBank accession numbers of human, animal and yeast ISWIs and DDT-domain proteins are *Homo sapiens* SNF2H (NP_003592), *Drosophila melanogaster* dISWI (NP_523719), *Saccharomy cescerevisiae* Isw2p (NP_014948), *Drosophila melanogaster* ACF1 (NP_536734), *Drosophila melanogaster* NURF301 (NP_728507) and *Saccharomy cescerevisiae* Itc1 (NP_011382).

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Figure S1. In planta interaction of DDP2 and CHR11.
The BiFC assay shows the in planta interaction between the CHR11 and the DDP2 full-length proteins in tobacco leaves. Note that both negative controls of DDP2-YC + YN and YC + YN-CHR11 showed no interaction signals.

Table S1. List of primers used in this study