AtFKBP53 is a histone chaperone required for repression of ribosomal RNA gene expression in *Arabidopsis*

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Chromatin structure is important for controlling gene expression, but mechanisms underlying chromatin remodeling are not fully understood. Here we report that an FKBP (FK506 binding protein) type immunophilin, AtFKBP53, possesses histone chaperone activity and is required for repressing ribosomal gene expression in *Arabidopsis*. The AtFKBP53 protein is a multidomain FKBP with a typical peptidylprolyl isomerase (PPIase) domain and several highly charged domains. Using nucleosome assembly assays, we showed that AtFKBP53 has histone chaperone activity and the charged acidic domains are sufficient for the activity. We show that AtFKBP53 interacts with histone H3 through the acidic domains, whereas the PPIase domain is dispensable for histone chaperone activity or histone binding. Ribosomal RNA gene (*18S rDNA*) is overexpressed when AtFKBP53 activity is reduced or eliminated in *Arabidopsis* plants. Chromatin immunoprecipitation assay showed that AtFKBP53 is associated with the *18S rDNA* gene chromatin, implicating that AtFKBP53 represses *rRNA* genes at the chromatin level. This study identifies a new histone chaperone in plants that functions in chromatin remodeling and regulation of transcription.

Keywords: *Arabidopsis*, immunophilin, chromatin, histone chaperone, ribosomal RNA, nucleosome assembly

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Introduction

Chromatin in all eukaryotes is composed of basic units called nucleosomes that consist of histones and DNA. The crystal structure shows that each nucleosome contains 146 bp of DNA wrapping around the central core histones (two copies of each of H2A, H2B, H3 and H4) [1-4]. However, the assembly and dynamics of chromatin and factors that regulate its structure are not well understood, especially in plant.

Histones are highly charged basic proteins and naturally tend to form complex aggregates that may not be suitable for chromatin assembly. Therefore a number of molecular chaperones are required to prevent them from making improper interactions and to facilitate histone deposition into chromatin structure [5-8]. Most chaperones for the core histones show preference for binding with either H3/H4 or H2A/H2B. For example, nucleosome

assembly protein 1 (NAP1) or NAP1-related protein, nucleoplasmin and nucleolin preferentially bind H2A/H2B [9-12], whereas anti-silencing function 1 (Asf1), histone cell-cycle-regulation-defective homolog A or chromatin assembly factor 1 (CAF-1) specifically interacts with H3/H4 [8, 13-15]. The deposition of histone variant, such as H2A.Z, requires specific proteins as well [16, 17]. Extensive biochemical experiments on the assembly of nucleosome *in vitro* were carried out to identify histone chaperones and these studies support the essential regulatory role of histone chaperones in mediating the deposition of histone onto DNA. For example, CAF-1, which is a heterotrimeric complex, is found to be generally associated with newly synthesized H3 and H4 and deposits histones on the replicating fork [18]. NAP1 is a histone chaperone that facilitates both the cytoplasm-nucleus transport of histone and its deposition into nucleosomes [19]. Recent studies also show new functions of histone chaperones in environmental adaptation in plant [20, 21].

It is widely believed that changes in chromatin structure alter gene expression [22-24]. Histone chaperones help maintain a delicate balance between nucleosome assembly and disassembly, thereby have profound influence on gene expression [16, 24, 25]. When chromatin

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is tightly packaged into the nucleosomes, it is not an active template for transcription. Factors that unravel nucleosomal structure often are involved in activating gene transcription. The activity of transcription generally correlates with low nucleosome density allowing for the passage of the transcriptional machine. For instance, yeast Asf1 plays a significant role in transcriptional activation of PHO family gene by the removal of nucleosomes from their promoter region [26, 27]. Deletion of *ASF1* or CAF-1 components led to global transcriptional misregulation, including both activation and repression, of genes functioning in a broad range such as chromatin, signal transduction and so on [28]. These studies suggest that histone chaperones function as active agents in the maintenance of chromatin dynamics and are global regulators of gene expression.

Identifying all the nuclear factors involved in nucleosome assembly will be the first step to understand the mechanism of chromatin remodeling and its effects on gene regulation. Although some histone chaperones are identified, many remain to be uncovered. As a large group of protein folding catalysts, some immunophilins have recently been shown to have a role in remodeling chromatin. The first nuclear FKBP protein, Fpr3, was isolated from *S. cerevisiae* and shown to interact with histone H2B [29]. Furthermore, SpFkbp39p and Fpr4 were identified as acidic histone chaperones in *S. pombe* and *S. cerevisiae*, respectively [7, 30]. In plants, *Arabidopsis* AtCYP71, a member of cyclosporine A binding proteins (cyclophilins, CYPs), was also shown to interact with histone H3 and regulate gene expression pattern that determines the development of *Arabidopsis* [31]. Analysis of these factors suggested that nuclear immunophilins might contribute to the dynamics of chromatin complex assembly and/or disassembly because they interact with basic chromatin components.

Results

Domain structure of the AtFKBP53 protein

AtFKBP53 is encoded by a single gene locus (At4g25340) in the *Arabidopsis* genome. This protein is a member of the FKBP-type immunophilin family in *Arabidopsis* [32]. It belongs to the multidomain immunophilins that contain one or more other identifiable protein motifs in addition to the catalytic peptidylprolyl isomerase (PPIase) domain. The AtFKBP53 protein contains a single FKBP domain at the C terminus and a highly charged N terminus with several regions enriched in acidic and basic amino acid residues. AtFKBP53 has three acidic regions enriched with glutamate and aspartate residues and one basic region rich in lysine residues (Figure 1). Sequence

alignments show that AtFKBP53 contains similar domain structure as yeast SpFkbp39 [7]. AtFKBP53 and SpFkbp39 share the positively charged domain in the middle of the proteins, which is different from previously characterized histone chaperones containing only acidic domains. A BLAST search using AtFKBP53 protein as a query identified homologues in rice, with similar acidic, basic and FKBP domains. The similar domain structure indicates that this type of histone chaperone may be highly conserved and have similar roles in different organisms.

Subcellular localization and expression pattern of AtFKBP53

The similarity of protein structure between AtFKBP53 and yeast SpFkbp39 suggests that AtFKBP53 may have similar function with SpFkbp39. As a step toward understanding the function of AtFKBP53, we determined the subcellular localization of AtFKBP53. We fused *AtFKBP53* coding sequence with RFP and transformed the DNA construct into onion epidermal cells. Subcellular localization was determined using epifluorescence microscopy. As a control, we bombarded onion epidermal cells with vector containing only DNA sequence encoding the RFP protein. We found that red fluorescence was distributed in both the cytoplasm and the nucleus (data not shown). When the cells were transformed with the AtFKBP53-RFP protein fusion construct, the red fluorescence was predominantly detected in the nucleus (Figure 2A and 2B), suggesting that AtFKBP53 is localized in the nucleus.

It was reported that *AtFKBP53* was broadly expressed in all the tissues examined, such as leaves, flowers, stems and roots [32]. To examine the detailed expression pattern of *AtFKBP53* gene, we fused *AtFKBP53* promoter with the GUS reporter and transformed it into *Arabidopsis*. Transgenic plants expressing *AtFKBP53::GUS* were analyzed and showed strong GUS staining in root apical meristem region and pollen (Figure 2C-2E). We compared microarray data in Genevestigator and found that *AtFKBP53* expression level in the root tip is remarkably higher than other tissues examined (Figure 2F).

Histone chaperone activity of AtFKBP53

Nuclear localization of AtFKBP53 and structural similarity with other histone chaperones such as Spfkb39 prompted us to examine whether AtFKBP53 can exert its effect as a histone chaperone and function in chromatin remodeling. We expressed and purified the recombinant proteins, SpFkbp39-His, GST, GST-AtFKBP53 and truncated forms of AtFKBP53 from *E. coli*. The truncated forms include GST-AtFKBP53-N1, GST-AtFKBP53-N2

Figure 1 Sequences alignments of AtFKBP53, AtFKBP43 and SpFkbp39. Amino acids of full-length proteins of AtFKBP53, AtFKBP43 and SpFkbp39 were used for the alignment. E-, D- or K-enriched regions of AtFKBP53 are highlighted by boxes. Spfk39 is SpFkbp39, Atfk53 is AtFKBP53 and Atfk43 is AtFKBP43. Consensus amino acids among these sequences are colored.

and GST-AtFKBP53-C1, structures of which are shown in Figure 3A. Equal molar amount of protein was used for all proteins to perform plasmid supercoiling assay. SpFkbp39 was taken as a positive control, and GST as a negative control. Enhanced production of supercoiled plasmid was observed in the presence of AtFKBP53, as in the presence of SpFkbp39, suggesting that AtFKBP53, like SpFkbp39, has histone chaperone activity. When

GST or GST-AtFKBP53-C1 was used in this assay, no supercoiled plasmid was detected. However, when GST-AtFKBP53-N1 or GST-AtFKBP53-N2 was added in this assay, supercoiled plasmid was again observed (as shown in Figure 3B). Taken together, our study data indicate that AtFKBP53 possesses histone chaperone activity. The acidic domains are required and sufficient for histone chaperone activity.

Figure 2 Subcellular localization of AtFKBP53 protein and expression pattern of the *AtFKBP53* gene. **(A and B)** Nuclear localization of the AtFKBP53-RFP fusion protein transiently expressed in onion epidermis. **(A)** Bright field of an onion epidermis cell. **(B)** Red fluorescence of an onion epidermis cell. **(C-E)**, Expression pattern of *AtFKBP53::GUS* fusion gene. **(C)** GUS activity in the root apical region. **(D)** GUS activity in the flower. **(E)** GUS activity in the pollen. **(F)** *AtFKBP53* gene expression pattern. Histograms represent expression level of *AtFKBP53* gene in different tissues. Bars represent standard deviation. The graph was generated based on the microarray data from Genevestigator (<https://www.genevestigator.com/gv/index.jsp>). Scale bars: 10 μ m in **(A-B)**, 0.2 mm in **(C)** and **(E)**, 1 mm in **(D)**.

AtFKBP53 interacts with histone H3

We showed that AtFKBP53 has histone chaperone activity and facilitates deposition of histones to DNA. To further understand how AtFKBP53 serves as a histone

chaperone and the kind of histones it can directly interact with, we performed an *in vitro* pull-down assay using purified protein GST-AtFKBP53 with core histone proteins. GST-AtFKBP53 or GST protein was immobilized to the

Figure 3 AtFKBP53 protein domains and histone chaperone activity assay. **(A)** Schematic presentation of SpFkbp39, full-length and truncated forms of AtFKBP53. **(B)** Plasmid supercoiling assay for different proteins. The names of protein samples are labeled under each lane.

glutathione-Sepharose beads that were further incubated with histones. The beads were washed and analyzed by SDS-PAGE. The gel was stained by Coomassie blue, which showed an obvious 17 kDa protein precipitated specifically by AtFKBP53, not by GST. We further confirmed the precipitated protein by western blot using histone H3 antibody. We found that histone H3 was associated with AtFKBP53 *in vitro*, but not with GST protein. In addition, our data earlier also showed that the acidic domain is sufficient for histone chaperone activity, suggesting possible association of histone H3 directly to the acidic region of the AtFKBP53 protein. Further *in vitro* pull-down assay using truncated AtFKBP53 proteins detected histone H3 interaction with GST-AtFKBP53-N1 and GST-AtFKBP53-N2, but not with GST-AtFKBP53-C1. This confirmed that the acidic domains are required and sufficient for binding histone H3 (Figure 4A and 4B).

Suppression of AtFKBP53 gene enhances 18S rRNA expression

To determine the biological function of AtFKBP53, we isolated one T-DNA insertion mutant of *AtFKBP53*

gene from ABRC seed center. Genomic DNA analysis shows that the T-DNA was inserted between the first exon and first intron (Figure 5A). We named this T-DNA line as *fkbp53-1*. RT-PCR analysis showed that mRNA level of *AtFKBP53* was reduced in the *fkbp53-1* mutant (Figure 5B). The *fkbp53-1* mutant does not show obvious developmental defects under normal conditions. We further applied RNA interference technique to silence *AtFKBP53* gene expression in transgenic plants. The *AtFKBP53* expression was essentially eliminated in the RNA interference line. We chose one RNAi line named *fkbp53-2* mutant for further studies (Figure 5C). The *fkbp53-2* mutant plants, like the T-DNA insertion line, show no developmental defects.

It was previously reported that SpFkbp39 in yeast is not an essential gene but required for repressing the ribosome RNA gene expression in yeast [7]. If *AtFKBP53* plays a similar function as Spfkbp39 in yeast, we would expect that *fkbp53* mutants may have elevated levels of 18S rRNA. Indeed, in both *fkbp53-1* and *fkbp53-RNAi*

Figure 4 The AtFKBP53 protein interacts with histone H3. **(A)** Coomassie blue staining of GST-AtFKBP53 and GST-truncated forms (N1 and N2) that were expressed and purified from *E. coli* and used to examine the interaction with the H3 peptide in **(B)**. **(B)** The H3 peptide pulled down by full-length or truncated forms (N1 and N2) of AtFKBP53 was analyzed by western blot using anti-H3 antibody.

Figure 5 The *fkbp53* mutants and 18S *rRNA* expression analysis. **(A)** Position of *fkbp53-1* T-DNA insertion. **(B)** *AtFKBP53* expression in wild-type and *fkbp53-1* mutant. **(C)** *AtFKBP53* expression in transgenic plants of RNAi empty vector and *AtFKBP53* RNAi construct, *fkbp53-2* was used to represent the transgenic plant from RNAi construct. **(D)** 18S *rDNA* expression in wild-type and *fkbp53-1* mutant. **(E)** Quantitative PCR assay of expression level of 18S *rDNA* in wild-type, *fkbp53-1* and *fkbp53-2* mutants. Bars represent standard deviation.

mutants the 18S *rRNA* level was higher as compared with that of wild-type plants (Figure 5D). We also quantified the expression 18S *rRNA* levels by real-time quantitative PCR. The results showed that 18S *rRNA* level was increased in both *fkbp53-1* and *fkbp53-RNAi* (Figure 5E).

AtFKBP53 associates with 18S *rDNA* chromatin

AtFKBP53 was localized in the nucleus and *in vitro* pull-down assay showed that *AtFKBP53* physically interacts with histone H3. To test the possibility that *AtFKBP53* interacts with *rRNA* gene chromatin and represses *rRNA* gene expression, we expressed *AtFKBP53*-GFP fusion in transgenic *Arabidopsis* plants. The fusion protein was detected by western blot using GFP antibody and by examining the GFP (Figure 6A and 6B). We used the ChIP technique to examine the association of *AtFKBP53* with *rDNA* using three independent transgenic plants expressing *AtFKBP53*-GFP. The ChIP assay of these lines showed that *AtFKBP53* associates with 18S

Figure 6 Association of *AtFKBP53* with 18S *rDNA* chromatin. **(A)** *AtFKBP53*-GFP fluorescence. **(B)** Western blot analysis of total protein in wild-type and transgenic plants using anti-GFP antibody. **(C)** ChIP results from three independent transgenic lines of 35S-*AtFKBP53*-GFP. Samples were from leaves of 28-day-old transgenic plants. The input is chromatin before immunoprecipitation. 1-3, three lines of 35S-*AtFKBP53*-GFP plants; -AB, ChIP product with no antibody; GFP-AB, ChIP product with antibody to GFP. Scale bar, 10 μ m in **(A)**.

rDNA *in vivo*, whereas AtFKBP53 did not associate with other loci we examined, such as Knotted1-like (*KNAT1*), CYCLIN D (*CYCD*) and APETALA1 (*API*) (Figure 6C; data not shown).

Discussion

We report that a nuclear FKBP-type immunophilin, AtFKBP53, is a functional histone chaperone that physically interacts with histone H3 and is possibly involved in H3/H4 deposition to the nucleosome. Importantly, AtFKBP53 can interact with ribosomal DNA chromatin and regulate its gene expression, implicating AtFKBP53 in nucleosome assembly and gene regulation in plants.

The physiological functions of immunophilins, despite numerous biochemical analyses, are not well understood in plants or in other eukaryotic organisms. AtFKBP53 is a multidomain immunophilin that contains highly charged residues. We have shown here that AtFKBP53 functions as a histone chaperone through the acidic regions. Other histone chaperones reported to date all have acidic regions that are likely to be responsible for binding to histones that are highly basic proteins [30, 33–37]. For example, both histone chaperones NAP-I and TAF-I interact with histones through an acidic region [38, 39]. In addition, the acidic domains in these proteins are also sufficient for histone chaperone activity, indicating that these negatively charged domains also directly participate in the “assembly process” of nucleosomes. Further structure studies will reveal the acidic structure and elucidate the action mode of histone chaperones. It is noteworthy that *Arabidopsis* AtFKBP53 not only contains the acidic regions but also contains a basic domain. Therefore, we conclude that AtFKBP53, like SpFkbp39 in yeast, may represent a novel type of histone chaperone in plants. However, the function of the basic region in these proteins remains to be revealed.

AtFKBP53 can physically interact with histones, mainly with H3. The interaction with histone H3 is an indicator for the function in the formation of an H3/H4 tetramer. Biochemical analysis has allowed for the separation of the nucleosome assembly reaction into two steps [40]. During the first step, the deposition of histones H3 and H4 to DNA occurs. The H3/H4 tetramer and DNA complex is a stable intermediate structure. In the second step, this preformed subnucleosome complex is converted to a mature nucleosome with the addition of two heterodimers of histones H2A/H2B. The binding of AtFKBP53 to histone H3 indicates that AtFKBP53 has a functional role in the first step, at least during formation of the nucleosome. Nevertheless, it is not ruled out that AtFKBP53 may function at the second step, because AtFKBP53 might interact with histones H2A/H2B, under

less stringent conditions or certain circumstances.

Structural change of chromatin in eukaryotic cells influences biological processes such as gene expression. The structure of chromatin is maintained in part by histone chaperones. However, the functional specificity of various histone chaperones is not well understood. It is speculated that changes in chromatin structure often bring about global changes in expression of many genes. For example, AtCYP71 in plants clearly has such a global role and the *cyp71* mutant plants showed a number of developmental defects caused possibly by de-repression of many genes [31]. In this report, we show that AtFKBP53 associates with 18S rDNA chromatin and regulates the level of its expression. Significantly, the factors required for maintaining ribosomal gene expression remain largely unknown in plants. We therefore provide the first evidence showing that AtFKBP53 regulates *rRNA* gene transcription at the chromatin level in plants.

It is puzzling why deregulation of rRNA gene expression did not cause visible changes in plant development. It is possible that upregulation of rRNA levels is not sufficient to change the functionality of ribosomes because other components may be limiting. Alternatively, some other chaperones may functionally overlap with AtFKBP53. One such candidate is AtFKBP43, another immunophilin protein that has a similar structure as AtFKBP53. AtFKBP43 may thus have similar functions as AtFKBP53. The *fkbp43* mutant, like *fkbp53* mutant, did not show obvious defects under normal conditions (Li *et al.*, unpublished data). Further characterization of double mutant of *fkbp53 fkbp43* may clarify the biological function of these immunophilins. In addition, further studies on functional interactions between AtFKBP53 and other histone chaperones may reveal more complicated relationship among these factors. Various histone chaperones may be involved in distinct and yet overlapping processes that maintain and change chromatin structure. Isolation of more histone chaperones will lead to a better understanding of the mechanism underlying nucleosome assembly and regulation of gene expression.

Materials and Methods

Plant materials and growth conditions

The seeds of *fkbp53* (GenBank accession number SALK_091242; background, Col) were provided by the Arabidopsis Biological Resource Center (ABRC). Plants were grown in a greenhouse at 22 °C with a photon flux density of 180 mmol/m²/s under a 16:8 h light-dark cycle.

Plasmid construction and plant transformation

For protein expression, DNA fragments that encode for the AtFKBP53, as shown in Figure 2A, were PCR-amplified from the cDNA clone (U14536, from ABRC). Each forward primer car-

ries a *Bam*HI cutting site near its 5' end, whereas each backward primer carries an *Xho*I cutting site near its 5' end. For clones with N-terminal deletions (AtFKBP53-C1), a start codon was inserted following the coding sequence in the forward primer. These DNA fragments were digested with *Bam*HI and *Xho*I, and cloned into the pGEX-4T-3 vector, which encodes a GST tag at the N terminus of each fusion protein. For protein localization transient expression cDNA of *AtFKBP53* was cloned into pGDR vector by *Bgl*II and *Sal*I enzyme sites. For transgenic plant, cDNA of *AtFKBP53* without stop codon was cloned into pMD1 by *Xba*I and *Bam*HI sites. The constructs were transformed into *Arabidopsis* plants using the *Agrobacterium tumefaciens*-mediated floral dip procedure.

Purification of proteins

To purify AtFKBP53 and AtFKBP53 mutants, we introduced the expression vectors into *Escherichia coli* strain BL21. Cells were grown in LB medium supplemented with ampicillin (100 µg/ml) at 37 °C to OD600 0.5 and induced with 0.1 mM isopropyl- β -thiogalactopyranoside at 37 °C for 4 h. Cells from 1 liter cultures were then harvested and resuspended in 20 ml PBS. The cell suspensions were sonicated and the lysates were subjected to centrifugation at 10 000 rpm for 20 min. The supernatant was applied onto Glutathione Sepharose 4B beads prewashed with PBS. Purified proteins were resolved by SDS-PAGE and confirmed by western blot analysis.

Histone chaperone assay

The method was based on protocol described previously with slight modifications [7, 41]. We treated plasmid PUC19 (2 686 bp) with topoisomerase I (20 U/µg DNA; Invitrogen) for 30 min. HeLa core histones (4 pmol) were preincubated with various proteins, including GST, SpFkbp39, AtFKBP53 and AtFKBP53 mutants for 15 min, and then added the pretreated plasmid (0.4 µg) into each of the chaperone-histone mixture and incubated for an additional 60 min in 50 µl reaction buffer containing 0.15 M NaCl, 0.5 mM DTT and 20 mM Tris-HCl (pH 8.0), at 30 °C. The reactions were stopped by adding equal volume of stop buffer (glycerol 25%, 60 mM Tris-HCl (pH 8.0), 30 mM EDTA, 0.5% SDS) and were resolved in 1% agarose gel by electrophoresis followed by ethidium bromide staining.

Protein-protein interaction

In vitro pull-down assays were performed as described previously with modifications [31]. Purified recombinant GST-AtFKBP53, GST-AtFKBP53-N1, GST-AtFKBP53-N2, GST-AtFKBP53-C1 or GST (1.5 µg) was immobilized to glutathione Sepharose beads at 4 °C for 2 h in the A300 binding buffer. After the beads were washed with A300 buffer three times, 1.5 µg core histones (13-107; Upstate) was added and incubated for 4 h in A300 buffer. After the beads were extensively washed with A300 buffer, the histone H3 bound to the beads was determined by SDS-PAGE and immunoblotting. A300 buffer contained 25 mM Tris-HCl (pH 8.0), 10% glycerol, 0.01% Nonidet P-40, 1 mM EDTA and 300 mM NaCl. The histone H3 antibody (ab1791; Abcam) was used at a dilution of 1:5 000 for protein gel blot analysis.

RNA expression analysis

Total RNA was extracted by Trizol from plant materials, and cDNA was synthesized by SuperScript II RNA polymerase. RT-

PCR was conducted using Taq polymerase to examine the expression levels of genes. Real-time PCR was performed according to previous methods [31].

Detection of β -glucuronidase (*GUS*) activity

Plants were grown under long day condition in the greenhouse. Plant tissue was placed in X-Gluc solution [1 mg/ml 5-bromo-4-chloro-3-indolyl β -D-glucuronic acid (X-Gluc), 25 mM phosphate buffer (pH 7), 1.25 mM potassium ferricyanide, 1.25 mM potassium ferrocyanide, 0.25 mM EDTA and 0.25% Triton X-100] in a vacuum for 10 min at room temperature, and then incubated overnight at 37 °C, as previously described [42].

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) experiments were performed as described previously with some modification [43]. Approximately 1.5 g rosette leaves of 28-day-old *Arabidopsis* plants grown on soil were fixed with 0.25% formaldehyde (EMD) under vacuum for 30 min until the leaf turns to be transparent. The reaction was stopped by the addition of glycine. The leaf material was ground in liquid nitrogen, and chromatin was extracted. The chromatin was sonicated into fragments ranging from 400 bp to 1 kbp. The chromatin solution was precleared with Protein A agarose beads/salmon sperm DNA slurry (16-157; Upstate). The sonicated chromatin was immunoprecipitated with 8 µl of anti-GFP (Santa Cruz). Cross-linking was reversed in the immunoprecipitated complexes by the addition of NaCl to a final concentration of 200 mM and incubation at 65 °C for 6 h to overnight. The DNA was extracted from the immunoprecipitates by treatment with 20 µg proteinase K, and purified by phenol-chloroform extraction and precipitation by ethanol and 15 mg/ml glycogen. Co-precipitated DNA was dissolved in 20 µl TE, and typically 1 µl was used for PCR analyses. DNA was amplified using primers specific to region of 18S rDNA. Thirty cycles were used to amplify PCR fragment using Ex-Tag polymerase (Takara, Kyoto).

Transient expression assays in onion epidermal cells

For particle bombardment, all plasmids were amplified in *E. coli* strain and purified using plasmid midi or mini kits according to the manufacturer's instructions (Qiagen, Valencia, CA). Particle bombardment assays were performed on the epidermal peels from onion bulb scales. Onion epidermis was bombarded with gold particles coated with plasmids using a PDS-1000/He Biolistic particle delivery system Bio-Rad (Hercules, CA). Bombarded tissues were incubated in MS medium for 24-48 h in dark. Microscopy GFP and red fluorescence protein (RFP) were visualized using a Leica MZ FLIII fluorescence stereomicroscope equipped with GFP and RFP filters (Leica, Wetzlar, Germany) [44, 45].

Accession numbers

Sequence data for *AtFKBP53* and *18S rDNA* can be found in GenBank under the nucleotide accession numbers At4G25340 and X16077, respectively.

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