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Analysis of ATH1 Transcription Activity and Co-IP of ATH1 Protein Interaction with OFP1

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Abstract ATH1 has multifaceted functions in regulating growth and development, and possibly regulate development by interacting with OFP1 protein. However, the protein interaction of ATH1 with OFP1 has not been further confirmed, and the transcription activity of ATH1 has not been determined. In this work, the interaction ATH1 with OFP1 was further confirmed by using Co-IP, and the transcription activity of ATH1 was determined by using Protoplast transfection system. The results showed that the ATH1-OFP1 protein complexes could be formed *in vivo*, and ATH1 was a transcription inhibitory factor, and OFP1 could enhance the transcription inhibitory activity of ATH1. This study will provide molecular basis for analysis of the regulatory functions of OFP-TALE complexes in plant development, which is great significance for improving important plant traits.
Keywords ATH1; Transcriptional activity; Protein interaction; Co-IP

The TALE family proteins contain two subclasses KNOX and BELL. Recent studies on plant developmental biology showed that KNOX and BELL can regulate organogenesis and morphological development of plants in many aspects. For example, KNOX can regulate stem elongation and leaf shape (Hake et al., 2004). It can also control the growth of plant stems (Uddin et al., 2015). The transition from vegetative growth to reproductive growth can be regulated by multiple BELL members. For example, in the growth of plant nutrition, ATH1 protein factor in the BELL family can inhibit stem elongation and regulate flowering transition (Gómez-Mena and Sablowski, 2008), and also promote stem development. In addition to ATH1, two other members of BELL protein play a similar role in regulating flowering stage of plants (Cole et al., 2006). In regulating the growth and development of plants, AtOFPs protein factors provide many functions. AtOFP1 is a multi-effect transcriptional regulator (Hackbusch et al., 2005), which can regulate the growth and development of plants in many aspects.

The results of previous studies showed that the possible interactions between OFP1 and ATH1 were preliminarily revealed by the matrix data obtained through prediction and large-scale yeast two-hybrid analysis and also in this reported network. Therefore, ATH1 may interact with OFP1 protein to regulate plant growth and development in many aspects (Hackbusch et al., 2005). However, the transcriptional activity of ATH1 has not been determined, and its interaction with OFP1 has not been confirmed by further experiments. To clarify the possible protein interactions of OFP1-ATH1 and further explore and study the regulatory functions of OFP and TALE-OFPs complex during plant development is an important scientific issue that needs to be solved urgently at present, which is of great significance for the genetic improvement of important traits in plants.

1 Results and Analysis

1.1 ATH1 transcriptional activity test and OFP1 regulation analysis of ATH1 transcriptional activity

Protoplast transient transfection system was adopted in this study, and the transcriptional activity of ATH1 protein was detected by the relative expression level of *GUS* gene. The transfection effector factor GD and trans effector factor LD-VP16 jointly activate the reporter gene, and the expression level of the reporter gene was relatively high in the transfection system used in this study (Figure 1). However, compared with the GD control, co-transfected

GD–ATH1 and Gal4[2X]:GUS showed lower expression levels (Figure 1). These results indicate that ATH1 protein plays a negative regulatory role in the transcription of downstream target genes.

In this study, *Arabidopsis thaliana* were transfected with factor GD--ATH1, LexA[2X]–Gal4[2X]:GUS, and OFP1 with HA under the control of CaMV 35S strong promoter. To test the effect of OFP1 protein on ATH1 protein transcriptional activity.

The experimental results showed that not only reflect the transcriptional activity of ATH1 factor, but also clearly showed the influence of ATH1 on transcriptional activity after the interaction between ATH1 and OFP1, and also indicate the protein interaction between the two *in vivo*, and OFP1 protein can significantly improve the transcriptional inhibitory activity level of ATH1 protein (Figure 1). In addition, the comparison and analysis of ATH1 protein amino acid sequence showed that it had a statistical model of transcriptional inhibition, namely LsLsLa structure. Therefore, the experimental results were consistent with the theoretical model and mutually verified the experimental results of this study.

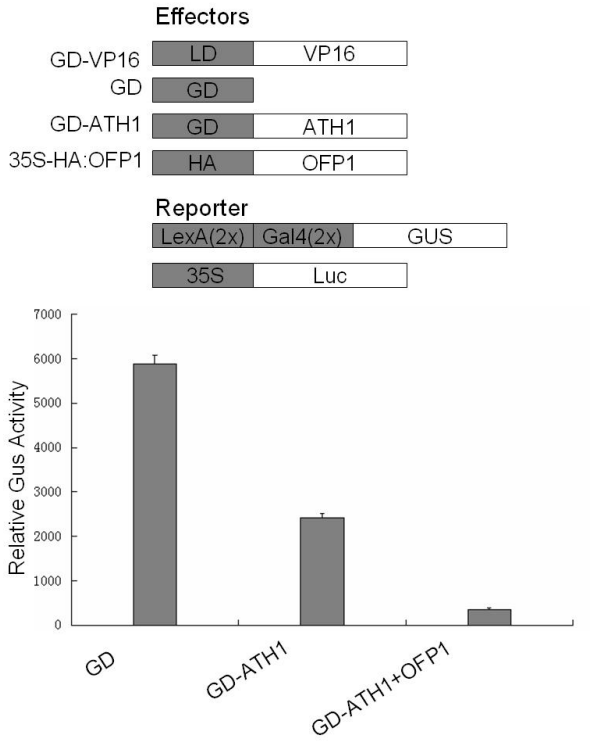

Figure 1 ATH1 is a transcriptional repressor that interacts with OFP1 *in vivo*

1.2 Co-IP determined the interaction between ATH1 and OFP1 proteins

The results of Co-IP showed that ATH1 protein and OFP1 protein interact *in vivo*. The extracted protein is divided into two parts, one is Input without any processing, and the other protein is added with corresponding antibody through synthetic antibody or transformation label, which is called IP. The interacting proteins were detected by FLAG antibodies and co-expressed in protoplasts for Co-IP analysis, indicating that ATH1 protein and OFP1 protein interacted in plants (Figure 2).

2 Discussion

Compared with previous studies (Hackbusch et al., 2005; Uddin et al., 2015), in this study, the *in vivo* interaction of two proteins was determined by Co-IP method, rather than just a feasibility obtained by high-throughput data matrix. Furthermore, ATH1 was identified as a transcription protein with inhibitory effect by transient transfection of Arabidopsis protoplasts. Further *in vivo* protein interaction experiments showed that ATH1-OFP1 protein complex could be formed *in vivo*, but other protein factors may also be involved in the *in vivo* experiment, so this

effect is indirect. Just as the BELL-KNOX protein may be negatively regulated by OFP1. Therefore, the inhibition of ATH1-OFP1 by the transcriptional protein complex may be further amplified or reduced.

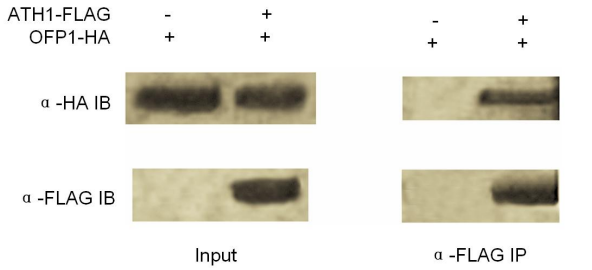

Figure 2 Co-expressing of ATH1- FALG and OFP1-HA

There is a sequence LSLSLA in the protein sequence of ATH1, which is a typical transcriptional inhibition domain structure located in the SKY-BELL region of the N-terminal of the protein. Transfection experiment and Co-IP experiments showed that AtATH1 could interact with AtOFP1, and was a transcriptional suppression. In terms of LSLSLA structure, its inhibition function mainly comes from 3 L structure, of which the first two L major inhibitory function, an auxiliary inhibitory effect, after the last one L mutation after inhibition function will weaken will not disappear. Studies on OFP1 showed that it was a transcription suppressor like ATH1, but it possessed both OVATE domain and three L structures. These results suggested that the negative regulation of OFP1 by target genes might be the result of resonance of multiple factors.

3 Materials and Methods

3.1 Material

All the experimental materials involved in this study were Columbia Arabidopsis (Col-0) type, including the materials of gene transformation types and mutants.

3.2 Methods

3.2.1 Transient transfection of protoplasts

PCR amplifiers with HA or GD tags and two 35S promoters linked to pUC19 (McCarthy et al., 2009; Zhang et al., 2018). To correspond to the HA label, pUC19 should be digested with endonuclease EcoRI enzyme before transfection by pZP211 plasmid.

35S: Gd-OFP1 was constructed into pUC19 vector for plasmid extraction and amplification experiments. In this transfection activity experiment, Gal4(2X):GUS and LexA(2X)-Gal4(2X):GUS, trans-acting factor LD-VP16, and effector protein factors ATH1 and OFP1 were used, as previously reported (Zhang et al., 2016). Protoplasts were transfected and extracted using the previously reported method (Zhang et al., 2016). Reporter factor and effector plasmid were analyzed, and EndoFree Maxi kit-Plasmid system was used. Student's T test was used as the statistical analysis method in this study, and two technical and three biological replicates were completed.

3.2.2 Co-IP

To accurately determine the expression level of the target protein, transient total proteins from Arabidopsis protoplasts or leaves were extracted for Input. anti-flag M2 agarose (Sigma) 45 μL treated material was taken, and total protoplasmic protein was added to the material, and incubated at low temperature on ice for 3 h to remove non-specific binding proteins, so as to competitively eluate flag factors and interact with protein factors. At the same time, 1.5 3xFLAG polypeptide was incubated at low temperature with the concentration of 0.5 mg/mL. After incubation at low temperature for 1.5 h, about 2 μg of HA antibody was added to it before treatment for 1~2 hours, and proteinAagarose was added before treatment. Then complete at least 3 washes, using the protein's buffer as a cleaning fluid. The protein buffer should be added to the supernatant with twice the concentration before 3 min variation at higher temperature.

Authors' contributions

ZLG is the experimental design and executor of this study, and has completed the analysis of the experimental results and the writing of the paper. ZXF is responsible for analyzing and proofreading data; CY, WGJ and ZSQ gave overall guidance. All authors read and approved the final manuscript.

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