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# Ovate family protein1 interaction with BLH3 regulates transition timing from vegetative to reproductive phase in Arabidopsis

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ABSTRACT

Three-Amino-acid-Loop-Extension(TALE) homeodomain transcription factor BLH3 regulates timing of transition from vegetative to reproductive phase. Previous preliminary results obtained using large-scale yeast two-hybrids indicate that BLH3 protein possibly interact with Ovate Family Proteins(OFPs) transcription co-regulators. Nevertheless, it is uncertain whether OFP1–BLH3 complex is involved in regulation of timing of transition from vegetative to reproductive phase in Arabidopsis.

The interaction between BLH3 and OFP1 was re-tested and verified by a yeast two-hybrid system. We found that the BLH3–OFP1 interaction was mainly mediated through the BLH3 homeodomain. Meanwhile, this interaction was further confirmed by bimolecular fluorescence complementation (BiFC) *in vivo*. Further, by establishing protoplast transient expression, we discovered that BLH3 acts as a transcriptional activator, whereas OFP1 functioned as a repressor. The interactions between OFP1 and BLH3 can reduce BLH3 transcriptional activity. The *ofp1* mutant lines and *blh3* mutant lines, *OFP1* overexpress lines and *BLH3* overexpress lines can both influence timing of transition from vegetative to reproductive phase. Furthermore, *35s:OFP1/blh3* plants exhibited flowering and leaf quantity similar to that of the wild-type controls. *35s:BLH3/ofp1* plants flowered earlier and had less leaves than wild-type controls, indicating that OFP1 protein might depend partially on BLH3 in its function to regulate the timing of transition from vegetative to reproductive phase. These results support our assumption that, by interacting with OFP1, BLH3 forms a functional protein complex that controls timing of progression from vegetative to reproductive phase, and OFP1 might negatively regulate BLH3 or the BLH-KNOX complex, an important interaction for sustaining the normal transition from vegetative to reproductive phase.



## 1. Introduction

Regulation of the timing of transition from vegetative to reproductive phase is an important biological process, which controls the point in time during development when a vegetative meristem changes and becomes an inflorescence or floral meristem and the rate at which the change occurs.

BLH3 is one of 13 BEL1-like (BLH or BELL) members in Arabidopsis. Several BELL members have been shown to govern the transition from the vegetative to the reproductive phase in plants. For example, plants overexpressing BLH6 flower later, whereas BLH3 overexpression induces early flowering [1].

OVATE genes encode a protein with an approximately 70-aa C-terminal domain which is conserved in tomato, rice, and Arabidopsis [2]. In Arabidopsis, 18 genes are predicted to encode the OVATE domain proteins. Heretofore, only a few AtOFPs have been shown to regulate plant growth and development.

The representatives of the OFP family are plant-specific proteins, indicating a close functional connection with TALE homeodomain proteins [3]. In a previous study, the BLH1-KNAT3 protein complex was negatively regulated by AtOFP5 protein during early embryo sac development [4]. More recently, a report revealed that AtOFP4

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participated in the regulation of secondary cell wall formation by interacting with KNAT7 [5].

The protein interactions of Arabidopsis TALE homeodomain proteins were systematically analysed by using the yeast two-hybrid assay, which evidenced a complicated regulatory interaction network among OFP, KNOX, and BLH in Arabidopsis; the interactions of several OFPs with BLH3 were also reported [3]. However, the findings on some interactions in the network have been invalidated by using other methods to establish protein interactions. Furthermore, at present, the information concerning the biological development roles of OFPs or complex of TALE-OFPs is still scarce.

Here, the interaction of OFP1 and BLH3 was verified in *Arabidopsis thaliana*; BLH3 acted as an activator, whereas OFP1 functioned as a repressor. Moreover, The research results suggested that OFP1 plays an important role in regulating time of transition from vegetative to reproductive phase by interacting with BLH3, supporting the extensive information regarding OFP regulatory functions in plants.

## 2. Materials and methods

### 2.1. Plant materials

All mutants, the wild-type and transgenic lines, were in the background of ecotype Columbia Arabidopsis (Col-0). Seeds were germinated and grown on 1/2 Murashige and Skoog basal medium containing vitamins, and was cold-treated in the dark for 48 h, and incubated at 22 °C under the conditions of a 14 h light/10 h dark photoperiod.

The mutant allele of *BLH3*, GK-961A08, which is a T-DNA insertion, designated as *Atblh3-1*, was evaluated by consulting the SIGnal database, and seeds were ordered from the NASC(European Arabidopsis Stock Centre). An *BLH3* gene-specific primer (5′-ATGGCTGTGTATTACCCTAAT AGTGTC-3′,5′-TTAGACAA-CAAAGTCGTGTAATTGATG-3′) and the T-DNA-specific primer (5′-ATAATAACGCTGCGGACATCTACATTTT -3′) were used in PCR analysis. The insertion site of T-DNA was identified by checking nucleic acid sequences.

The floral dip method was utilized for transgenesis in wild-type Arabidopsis plants background, as described earlier by Clough and Bent (1998) [6]. At least five transgene lines were identified and confirmed in minimum T3 generations.

### 2.2. Plasmid construction for protein–protein interactions in yeasts

The Open Reading Frame(ORF) of *OFP1* and *BLH3* was cloned in wild-type Arabidopsis cDNA. The homeodomain and SKY-BELL domain were divided according to Cole method [1] and using tools at http://smart.embl-heidelberg.de/.The homeodomain and SKY-BELL fragments were obtained by amplification of BLH3 plasmids(5′-TCGTTACGTTGGCCTTGGAA-3′, 5′-CTCCCCCAAT-CAACTGGCAT-3′,and5′-ACGGAAACGTAACGGTGTCA-3′, 5′-ACCCTGATGTCTCGTAACGG-3′). Fusion plasmids of pGBKT7-AtOFP1 were transformed into the yeast strain Y2HGold, and BLH3 cDNA was inserted into pGADT7. Their interaction was tested on SD medium(-Trp, -His,-Trp, -Ade) after selection on SD (-Trp, -Leu) medium. The known interaction between pGBKT7-p53 and pGADT7-SV40 plasmids was used as a positive control. The yeast cells harbouring the pGBKT7-Lam and pGADT7-SV40 plasmids were used as the negative control.

### 2.3. Bimolecular fluorescence complementation (BiFC) assays

The fusion proteins of AtBLH3-YFPN and AtOFP-YFPC under 35S promoter regulation were obtained by using N-terminal of EYFP vectors and the C-terminal of EYFP vectors [7].

After amplifying cDNA, the full sequences of AtOFP1 and AtBLH3 were sub-cloned into the pSAT6-nEYFP-N1 vector and pSAT6-cEYFPN1 vector, respectively. The resulting constructs were used for transient assays by polyethylene glycol transfection of Arabidopsis protoplasts, as described by Yoo et al. [8]. Transfected cells were imaged by using a Leica TCS SP2 confocal spectral microscope imaging system.

### 2.4. Plasmid constructions and protoplast transfection analysis in Arabidopsis

The constructs of OFP1 prom: GUS,35S:GD-OFP1 and 35S:HA-OFP1 have been outlined in an earlier study [5,9].

pUC19 with two 35S enhancer promoters was connected with a PCR fragment, which was cloned in a frame with a GD tag or a N-terminal HA tag [10,11]. The pUC19 vector was digested with the EcoRI restriction enzyme and was inserted into the binary vector pZP211 via plant transformation for corresponding constructs with the HA tag [12].

35S:GD-OFP1 constructs in the pUC19 vector, which were used for protoplast transfection and plasmid DNA extraction. The effector of AtOFP1 genes, LD-VP16 transactivator, and the reporter genes LexA(2X)-Gal4(2X):GUS and Gal4(2X):GUS were utilized, as described in previous reports [5,9]. The abovementioned methods were employed for the extraction of protoplasts and execution of transfection experiments [11,13]. Student's t-test was applied for statistical analyses. At least three replicates were performed in all transfection analyses, which were repeated at least twice.

### 2.5. Reverse transcription PCR and GUS expression assay

RNA samples from fresh Arabidopsis tissue were extracted by using RNA extraction kit (Takara) according to the manufacturer's instructions, and reverse transcriptase by the RT kit (Takara). *AtBLH3*-specific primers (5′-ATGGCTGT GTATTACCCTAATAGTGTC-3′, 5′- TTAGACAACAAAGTCGTGTAATTGATG-3′). *ACTIN* gene (At2g37620) was regarded as a positive control(primers 5′-C CAGAAGGATGCATATGTTGGTGA-3′, 5′-GAGGAGCCTCGGTAA-GAAGA-3′).

The prom *BLH3*:GUS for plant transformation was generate by *BLH3* promoter, a 742-bp genomic fragment upstream of the *BLH3* coding region was fused to the GUS reporter gene. The GUS expression activity of 7- and 10-day-old Arabidopsis tissues was detected in a buffer solution (pH 7.0), which contained 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-GLUC), 0.1% Triton X-100, and 0.5 mM potassium ferricyanide, and the tissues were incubated for 1–12 h at 37 °C. The tissue under investigation was observed via a Leica DM6000 microscope.

## 3. Results

### 3.1. BLH3 interaction with OFP1

The complicated interaction network among the proteins, i.e., OFP–KNOX–BELL, indicated potential interactions of BLH3 with some OFPs, including those with OFP2, OFP3, OFP4, and OFP5, beside that with OFP1 [3].

At present, only the insertion mutants of OFP1 and OFP4 among OFP members are used. AtOFP1 is localized in the nucleus and regulates AtGA20ox1, which is a gene encoding the key enzyme in gibberellin(GA) biosynthesis [9]. Further, GA can regulate growth and development in Arabidopsis, including the control over the timing of transition from vegetative to reproductive phase [14–16].