

Structure of florigen activation complex

Florigen, a mobile floral induction factor encoded by *FLOWERING LOCUS T* (*FT*) and its homologs, is thought to form a complex with the bZIP transcription factor FD to activate floral identity genes such as *APETALA1* (*AP1*) at the shoot apex. However, many details of the molecular function of florigen remain unclear. Here, we present the 2.4 Å crystal structure of a rice floral induction complex, named the florigen activation complex (FAC), which consists of the rice florigen Hd3a, the FD homolog OsFD1, and a 14-3-3 protein [1]. Unexpectedly, 14-3-3 plays a key role by mediating an indirect interaction between Hd3a and OsFD1.

Florigen is produced in leaves and is transmitted through the phloem to the shoot apex, where it induces flowering. A number of recent reports have provided evidence that *Arabidopsis* FT protein (Hd3a in rice) is a key component of florigen [2]. In the shoot apical meristem, FT activates *AP1* transcription and induces flowering by interacting with the bZIP transcription factor FD, although the details of the interaction between FT and FD have not yet been clarified. In rice, the closest homolog of FD, OsFD1, has been identified on the basis of its function [1] and homology with maize DLF1. The FT-FD interaction is required for flowering, and phosphorylation of residue T282 in the C-terminal region of FD may be critical for this interaction and floral initiation.

We tested for a direct interaction between highly purified Hd3a and OsFD1 using three different methods, glutathione S-transferase (GST) pull-down assay, nuclear magnetic resonance (NMR) and isothermal titration calorimetry (ITC), but none was detected. This result was surprising; by analogy with the FT-FD interaction in *Arabidopsis*, Hd3a was expected to interact with OsFD1. Next, the known interaction between Hd3a and a rice 14-3-3 protein, OsGF14c (hereafter referred to as GF14) [3], was re-examined. A direct interaction was observed by GST pull-down and NMR experiments; NMR also revealed that Hd3a interacted directly with the closely related 14-3-3 protein OsGF14b. In an effort to reconcile these results, the amino acid sequence of the C-terminal region of OsFD1 was investigated, since the corresponding region of FD is important for the FT-FD interaction. One consensus sequence among the bZIP transcription factors that reportedly bind FT and its homologs was found to be R-x-x-(S/T)-A-P-F, which resembles the 14-3-3 protein-binding motif R-S-x-(pS/pT)-x-P. The presence of this sequence in OsFD1 raised the possibility that the interaction

between Hd3a and OsFD1 is indirect and is mediated by 14-3-3 proteins. A direct interaction between OsFD1 and GF14 was indeed observed in GST pull-down and yeast two-hybrid assays; moreover, this interaction depended on the phosphorylation of OsFD1 S192 (T282 in FD), which is partially consistent with a previous assertion of the importance of FD T282 phosphorylation for binding to FT. These results indicate that GF14 directly binds both Hd3a and phosphorylated OsFD1, and plays a critical role in the association between the two. This model may extend to the physical relationship between FT and FD in *Arabidopsis*, since FT and 14-3-3 proteins have been reported to interact.

To elucidate the structural basis of the interactions between these three proteins, we determined the crystal structure of an Hd3a-GF14-OsFD1 complex at 2.4 Å resolution (Fig. 1) using diffraction data collected at beamlines BL41XU and BL44XU. For structure determination, OsGF14c and a peptide comprising the nine C-terminal amino acids of phosphorylated OsFD1 were used; neither OsGF14b nor intact OsFD1 yielded diffraction-quality crystals. The complex is a heterohexameric composed of two molecules each of Hd3a, GF14 and OsFD1. Two Hd3a monomers abut the C-terminal regions of dimeric GF14, about 50 Å apart, resembling two equal weights on a balance, to form a thick and deep W-shaped structure (Fig. 1).

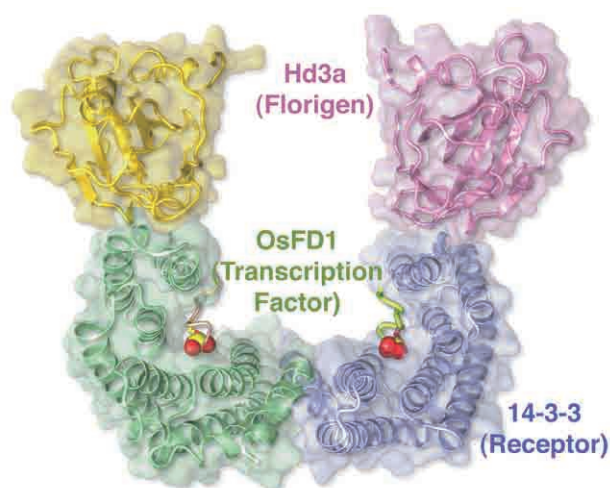


Fig. 1. Structure of the florigen activation complex (FAC). The FAC heterohexameric structure in a ribbon representation, composed of two Hd3a molecules (florigen; gold and magenta), a 14-3-3 dimer (receptor; dark green and blue), and two OsFD1 peptides (transcription factor; khaki and light green). Oxygen atoms in the phosphate groups of phosphoserine 192 of OsFD1 are visible as red spheres.

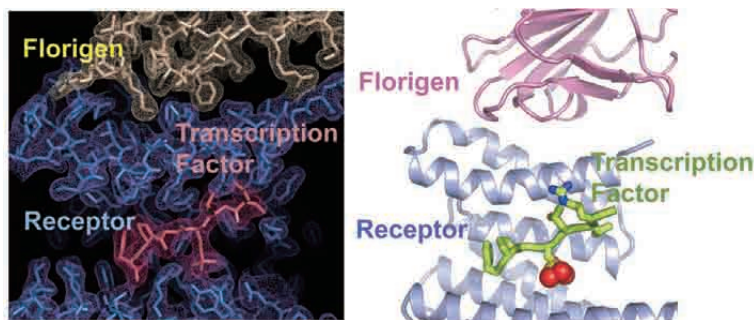


Fig. 2. Expanded view of the FAC structure shown by electron density map (left) and its corresponding ribbon representation (right). The binding sites in the receptor (blue) for florigen (gold and magenta) are more than 20 Å apart from those for the transcription factor (red and green), and no direct contact is observed between florigen and transcription factor.

The two corners at the inner base of the 'W' form positively charged pockets, and the S192-phosphorylated C terminus of OsFD1 binds to these pockets. The binding sites in GF14 for Hd3a are more than 20 Å apart from those for OsFD1 (Figs. 1 and 2), and yeast two-hybrid assays using GF14 mutants confirmed that the two partners are bound independently. Therefore, GF14 forms a stable complex with Hd3a and OsFD1 simultaneously, and mediates indirect binding between Hd3a and OsFD1. We named this complex the florigen activation complex (FAC).

On the basis of biochemical, biophysical and physiological experiments using rice cultured cells and

transgenic rice plants, we have shown that the florigen Hd3a forms a complex with 14-3-3 (GF14) and OsFD1, that is required for floral induction [1] (Fig. 3). We also found that 14-3-3 protein functions as a novel mediator of florigen function by bridging Hd3a and OsFD1. The Hd3a-14-3-3 complex may also interact with other bZIP transcription factors containing the R-x-x-(S/T)-A-P-F motif, to which 14-3-3 binds in the FAC. This could provide a mechanistic basis for the proposed participation of florigen in processes other than flowering [4]. In other words, 14-3-3 may not simply be a mediator of flowering but may play an even more central role as an intracellular receptor for florigen.

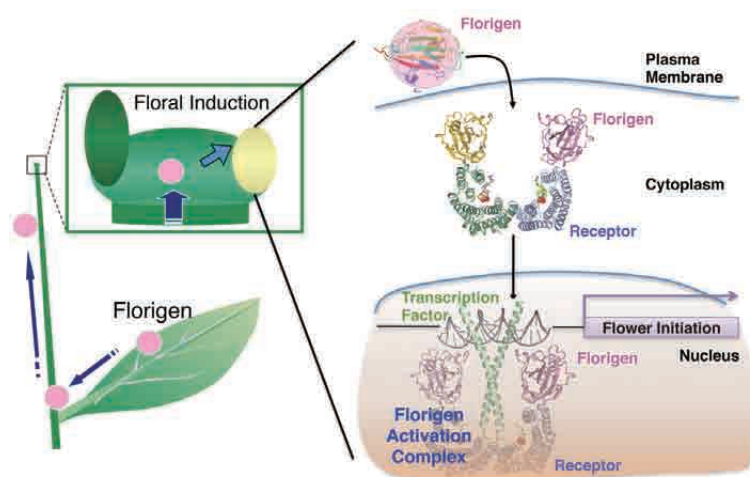


Fig. 3. Model of FAC formation. Florigen (Hd3a) is produced in leaves and is transmitted through the phloem to the shoot apex. Once florigen enters a shoot apical cell, it initially binds to the florigen receptor (14-3-3 proteins) in the cytoplasm. When the florigen-receptor complex enters the nucleus, it forms a complex with the transcription factor (OsFD1), which is retained in the nucleus and activates the flower initiation gene (*OsMADS15*) transcription, leading to floral induction.

Izuru Ohki^a, Ko Shimamoto^b and Chojiro Kojima^{a,c,*}

^a Lab. of Biophysics, Nara Institute of Science and Technology

^b Lab. of Plant Molecular Genetics, Nara Institute of Science and Technology

^c Institute for Protein Research, Osaka University

*E-mail: kojima@protein.osaka-u.ac.jp

References

- [1] K. Taoka, I. Ohki, H. Tsuji, K. Furuita, K. Hayashi, T. Yanase, M. Yamaguchi, C. Nakashima, Y.A. Purwestri, S. Tamaki, Y. Ogaki, C. Shimada, A. Nakagawa, C. Kojima and K. Shimamoto: *Nature* **476** (2011) 332.
- [2] S. Tamaki *et al.*: *Science* **316** (2007) 1033.
- [3] Y.A. Purwestri *et al.*: *Plant Cell Physiol.* **50** (2009) 429.
- [4] C. Navarro *et al.*: *Nature* **478** (2011) 119.