Discovery of Flowering Hormone (Florigen) Receptor and Its Crystal Structure

Florigen, a mobile floral induction protein, initiates the flowering process of activating floral identity genes. Many details of the molecular function of florigen remain unclear. In the present study, we found that the rice florigen Hd3a directly interacts with 14-3-3 (GF14) proteins, but not with the transcription factor OsFD1. We further determined the 2.4-Å crystal structure of a tripartite Hd3a-14-3-3-OsFD1 complex [1]. The determined crystal structure offers biological insights into 14-3-3 proteins and how they play a key role in mediating an indirect interaction between Hd3a and OsFD1. Our biochemical, biophysical, and physiological experiments using rice cultured cells and transgenic rice plants revealed that 14-3-3 proteins are intracellular receptors of florigen to activate floral identity genes.

Florigen is produced in leaves and 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 floral identity genes such as *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 based on its function and homology with maize DLF1 [1]. 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 for the floral initiation.

In the present study, a direct interaction between highly purified Hd3a and OsFD1 was tested using three different methods, but none was detected. On the other hand, a direct interaction between Hd3a and rice 14-3-3 protein GF14 was observed by GST pull-down and NMR experiments. One consensus sequence among the bZIP transcription factors, including OsFD1, 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 raises the possibility that the interaction between Hd3a and OsFD1 is indirect and is mediated by 14-3-3 proteins.

To elucidate the structural basis of the interactions between these three proteins, the crystal structure of a tripartite Hd3a-14-3-3-OsFD1 complex was determined at 2.4-Å resolution (Fig. 1), using the data recorded at the BL-5A, 17A and AR-NW12A. The binding sites in GF14 for Hd3a are more than 20 Å apart from those for OsFD1, and yeast two-hybrid assays using GF14 mutants confirmed that the two partners bound independently. Therefore, GF14 forms a stable complex with Hd3a and OsFD1 simultaneously, and mediates indirect binding between Hd3a and OsFD1. This complex was named Florigen Activation Complex (FAC).

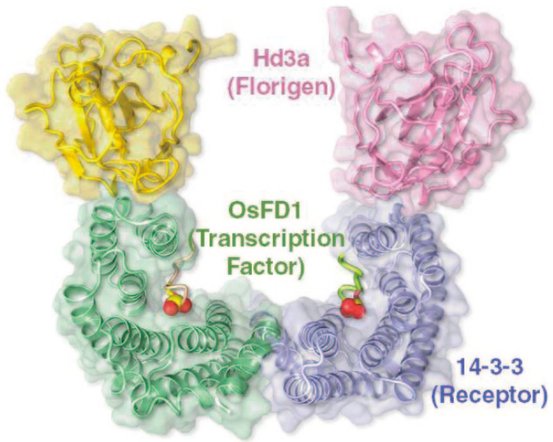

Figure 1
Structure of the Florigen Activation Complex (FAC). The FAC hetero-hexameric structure is represented as a ribbon, composed of two Hd3a molecules (florigen, gold and magenta), a 14-3-3 dimer (receptor, dark green and blue) and two OsFD1 peptides (transcription factor, khaki and light green). Oxygen atoms in the phosphate groups of phosphoserine 192 of OsFD1 are shown as red spheres.

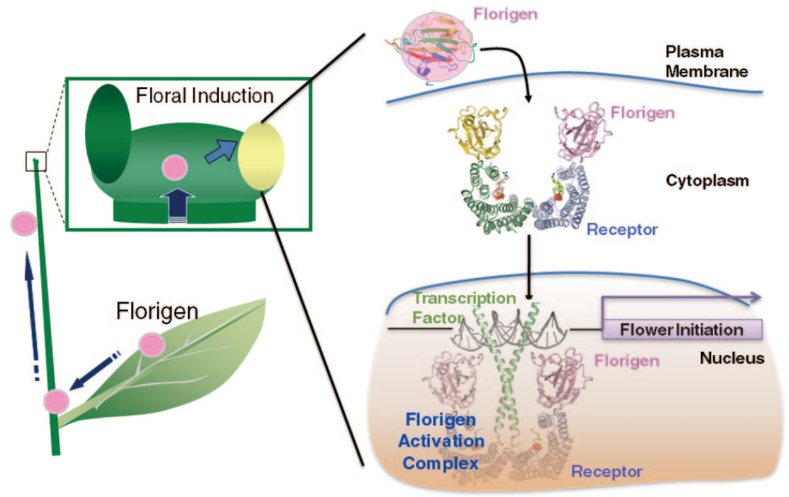

Figure 2
Model of the FAC formation. Florigen (Hd3a) is produced in leaves and is transmitted through the phloem to the shoot apex. Once florigen enters a shoot apical cell, it initially binds the florigen receptor (14-3-3 proteins) in the cytoplasm. When the florigen–receptor complex enters the nucleus, it forms a complex with the transcription factor (OsFD1), which is retained in the nucleus, and activates the flower initiation gene transcription, leading to floral induction.

Based on biochemical, biophysical, and physiological experiments using rice cultured cells and transgenic rice plants, it was shown that the florigen Hd3a forms a complex with 14-3-3 protein and OsFD1, and this complex formation is required for floral induction [1] (Fig. 2). In this model, the 14-3-3 proteins act as intracellular receptors for Hd3a (florigen), which is transported from the leaves to the shoot apex. Once Hd3a enters cells in the shoot apex, it initially binds the 14-3-3 proteins in the cytoplasm. At this stage of development, OsFD1 is being expressed in the shoot apex cells, and when the Hd3a-14-3-3 complex enters the nucleus, it forms a ternary complex with OsFD1, which will be retained in the nucleus. The formed FAC then activates the *AP1* transcription, leading to floral induction. By its nature, Hd3a-14-3-3 complex may also interact with other bZIP transcription factors containing the R-x-x-(S/T)-A-P-F motif, putative interaction that provides a mechanistic basis for the proposed participation of florigen in processes other than flowering, such as potato tuber formation [3]. Therefore, together with the discovery of the florigen receptor and the unraveling of the FAC structure, the possibility of manipulating florigen and other interacting regulators may pave the way for improving various important traits, such as early/late flowering, increased crop and fruit yields, and biofuel crop production.

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K. Taoka[1], I. Ohki[1], H. Tsuji[1], C. Kojima[1, 2] and K. Shimamoto[1] ([1]NASIT, [2]Osaka Univ.)