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Short Communication

Isolation and characterization of a *TERMINAL FLOWER 1* homolog, *RsTFL1*, from radish (*Raphanus sativus*)

Shosaku Oshima, Kazumasa Ikeda, Kazunari Nomura*

College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 252-8510 Japan
* E-mail: nkazu@brs.nihon-u.ac.jp Tel: +81-466-84-3514 Fax: +81-466-84-3625

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Abstract The gene *TERMINAL FLOWER1* (*TFL1*) regulates the floral phases and the inflorescence architecture of *Arabidopsis*. A *TFL1* homolog designated as Rs*TFL1* was isolated from radish (*Raphanus sativus*). The deduced amino acid sequence had 96% identity with TFL1—higher than the values obtained for other reported TFL1-like proteins. The genomic organization of *RsTFL1* is very similar to that of *TFL1* and consists of 4 exons and 3 introns. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis revealed that *RsTFL1*, like *TFL1*, is continuously expressed in both vegetative and reproductive tissues. *RsTFL1* was expressed throughout seed development until seed desiccation. *RsTFL1* mRNA was detected after seed imbibition, suggesting that the gene expression is initiated during germination. *In situ* hybridization analysis revealed that *RsTFL1* was expressed in the inflorescence meristem but not in the floral meristems. The expression was not limited to the inner cells of the inflorescence meristem, unlike the expression of *TFL1* in *Arabidopsis*. This expression pattern of *RsTFL1* may make it possible that radish maintains its indeterminate inflorescence.

Key words: Indeterminate inflorescence, radish, *Raphanus sativus*, shoot apical meristem, *TERMINAL FLOWER1*.

Arabidopsis and radish (*Raphanus sativus*) are both cruciferous plants that have indeterminate inflorescences with typical racemes. Radish has long been grown as a food crop worldwide and consists of extremely variable cultivars. In particular, in Southeast Asia (Japan, Korea, and China), the large-rooted form is one of the most important vegetables. In addition, some have importance in Chinese medicine, the seeds of some plants are used as oil sources, and the young siliques (pods) or leaves of some plants are consumed (Banga 1976). Radish shows considerable variations in temperature and photoperiod for flowering; however, little is known about the molecular mechanism underlying radish flowering. In *Arabidopsis*, *TERMINAL FLOWER1* (*TFL1*) represses floral transition and maintains the indeterminacy of the shoot apex; *tfl1* mutants flower early and terminate inflorescence development after the formation of a large compound flower (Shannon and Meeks-Wagner 1991; Alvarez et al. 1992). Conversely, the ectopic over-expression of *TFL1* prolonged all the growth phases, resulting in late flowering and more highly branched plants (Ratcliffe et al. 1998). On the other hand, the floral meristem identity gene *LEAFY* (*LFY*) imparts a floral fate to the lateral meristem (Weigel et al. 1992; Weigel and Nilsson 1995).

We recently isolated and characterized 2 *LFY*-like genes in radish, namely, *RsLFY1* and *RsLFY2* (Oshima and Nomura 2008). Moreover, since *TFL1* is known to play a key role in plant architecture in *Arabidopsis*, we isolated and characterized a *TFL1* homolog from radish that was designated as *RsTFL1* (*Raphanus sativus TFL1*) in order to understand the basic molecular mechanism of flowering in this plant. The cDNA sequence data have been deposited in the GenBank database (accession number: AB435524). In this study, the rat's tail radish cultivar "Pakki-hood" was used (Nomura et al. 1996). This particular variety does not require a chilling step to induce flowering. Total RNA extracted from radish shoot apices was used to isolate *TFL1*-like genes by reverse transcriptase-polymerase chain reaction (RT-PCR). The longest cDNA clone had a 531-bp open reading frame that was preceded by a 67-bp 5′-untranslated region. Two polyadenylation sites were found at positions 700 and 736 in the 3′-untranslated region. The cDNA showed extensive similarity to *TFL1*. PCR amplification of the corresponding genomic sequences revealed that *RsTFL1* contains 4 exons of 277, 66, 37, and 356 bp and 3 introns of 195, 227, and 90 bp (Figure 1A), and these were located at the same positions as in *TFL1* of *Arabidopsis* (Ohshima et al. 1997). The 531-bp open reading frame is predicted to encode 177 amino acid residues, and the deduced amino acid sequence has 96% identity with that of TFL1 (Figure 1B). Phylogenetic analysis revealed that *RsTFL1* and *TFL1* were positioned on the same branch

Abbreviations: SAM, Shoot apical meristem; PEBP, phosphatidylethanolamine-binding proteins
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of a phylogenetic tree, indicating that they have the closest relationship among the *TFL1*-like genes (Figure 1C). In *Arabidopsis*, *TFL1* and *FT* belong to the *PEBP* family (Bradley et al. 1997; Ohshima et al. 1997; Yeung et al. 1999; Kardailsky et al. 1999; Kobayashi et al. 1999). Both these genes regulate meristem identity and flowering time; however, despite the similarities in their sequences, they play antagonistic roles: *FT* induces floral

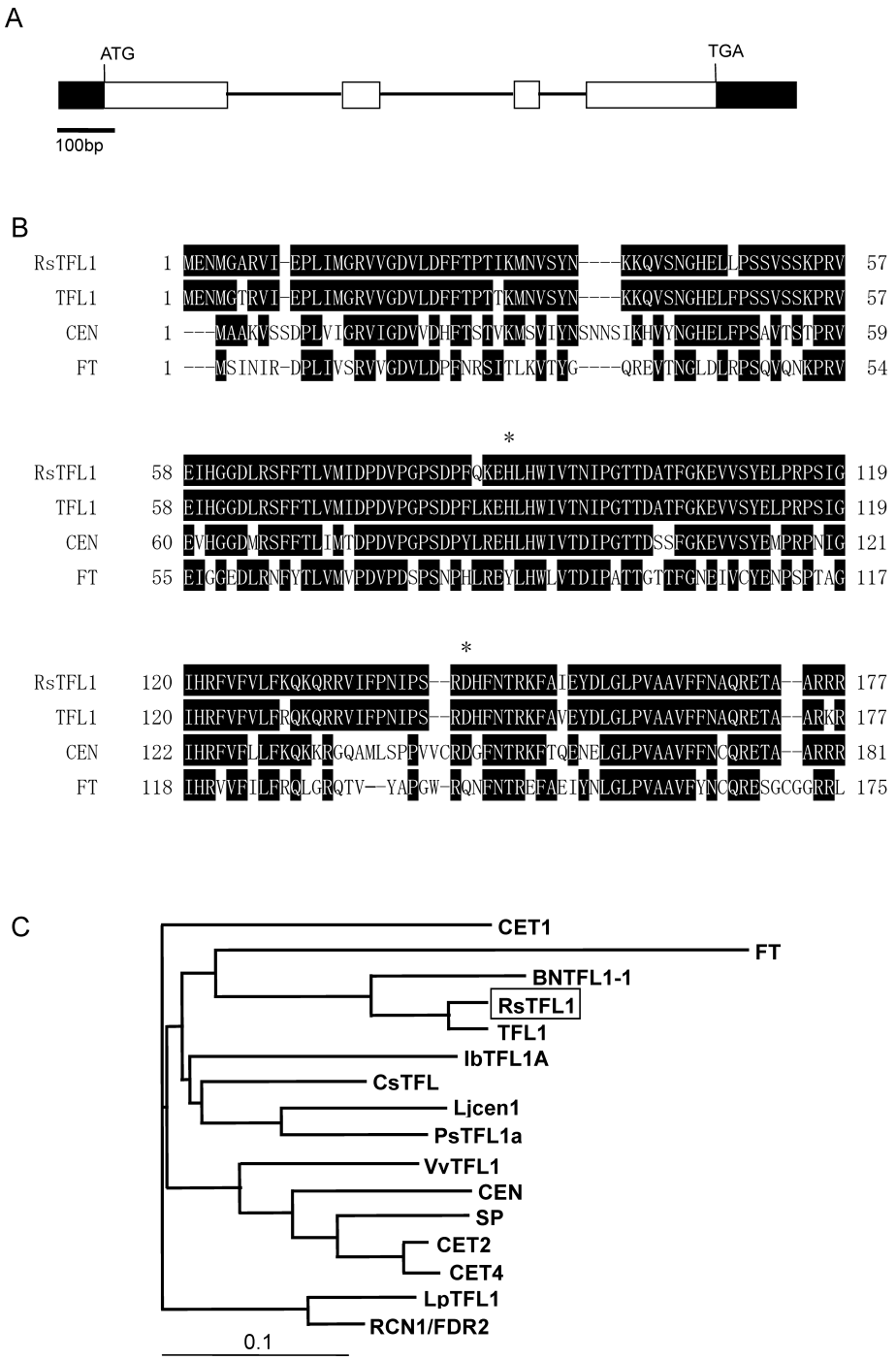

Figure 1. Genomic organization of *RsTFL1* and similarity of deduced protein with other plant PEBPs. (A) The structure of *RsTFL1* could be determined by comparison of cDNA clones with genomic DNA. Filled boxes, untranslated regions; open boxes, protein-coding regions; lines, introns. (B) Alignment of the predicted amino acid sequence of RsTFL1 with TFL1, CEN, and FT. Identical residues are in white text on black background. Dashed lines indicate gaps introduced to maximize alignment. Asterisks indicate amino acids that are likely to be most critical to the function of TFL1/CEN and FT (Hanzawa et al. 2005; Ahn et al. 2006). (C) Phylogenetic tree based on the predicted TFL1-like proteins and drawn by CLUSTALW. The lengths of horizontal lines are proportional to the similarity between predicted protein sequences. Accession numbers: CET1 (AF14259), FT (AB027504), BNTFL1-1 (AB017525), TFL1 (D87130), IbTFL1A (AJ888758), CsTFL (AB027456), Ljcen1 (AY423715), PsTFL1a (AY340579), VvTFL1A (DQ871591), CEN (S81193), SP (U84140), CET2 (AF145260), CET4 (AF145261), LpTFL1 (AF316419), RCN1/FDR2 (AF159882).

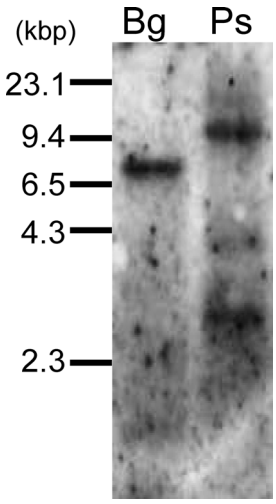

Figure 2. Southern blot analysis of the *RsTFL1* gene. *Raphanus sativus* genomic DNA was digested with *Bgl*II (Bg) and *Pst*I (Ps). The genomic sequence corresponding to *RsTFL1* included a site for *Pst*I. Full-length *RsTFL1* cDNA was labeled with digoxigenin and used as a probe. Hybridization and washing were carried out under high stringent conditions as described in the manufacturer's protocol (Roche).

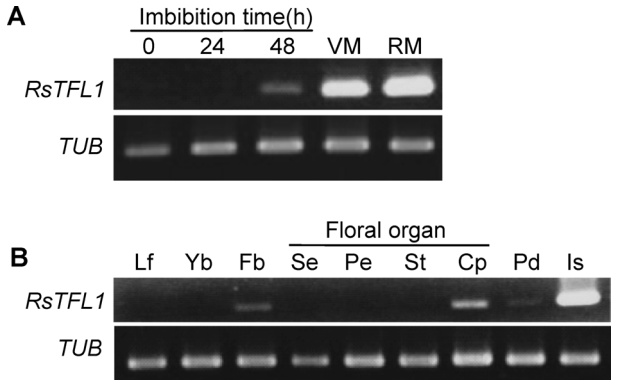

Figure 3. RT-PCR analysis of *RsTFL1* in radish. Total RNA was isolated from various tissues and germinating seeds with the RNeasy Mini Kit (Qiagen). Total RNA (1 μg) was treated with RNase-free DNase I (Invitrogen) to eliminate genomic DNA contamination. For RT-PCR, 1 μg of total RNA was used to generate first-strand cDNA in the SuperScript First-Strand Synthesis System for RT-PCR kit (Invitrogen). Primers for *RsTFL1* fragment amplification were F325 (5′-AGAGCACCTGCATTGGATCGTAAC-3′) and R3E2 (5′-CAGAAATTCAGAACATAAAAGCAAACATGGC-3′). The thermocycler program was 2 min/94°C; 30 cycles of 30 s/94°C, 30 s/60°C, 45 s/72°C; and a final extension of 5 min/72°C. A fragment of β-*TUBULIN* (*TUB*) gene was amplified from the same cDNA as a standard control to normalize the cDNA amount used in the RT-PCR. (Marks et al. 1987). (A) Expression of *RsTFL1* in imbibed seeds (0, 24, and 48 h) and shoot apices. (B) Expression of *RsTFL1* in various tissues: VM, vegetative meristem; RM, reproductive meristem; Lf, leaves; Yb, young floral buds; Fb, floral buds just before flowering; Se, sepals; Pe, petals; St, stamens; Cp, carpels; Pd, pedicels; Is, inflorescence stems.

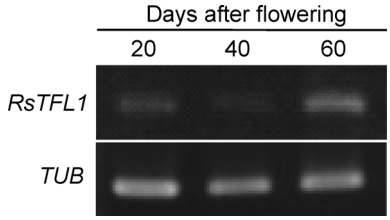

Figure 4. Expression of *RsTFL1* in developing seeds. Flowers were tagged on the day of flowering. Every 20 days during seed development (20, 40, and 60 d), siliques were harvested and seeds were picked out to extract total RNA. RT-PCR was performed using gene-specific primers. A fragment of *TUB* was amplified from the same cDNA as a standard control to normalize the cDNA amount used in the RT-PCR. (Marks et al. 1987).

transition whereas *TFL1* suppresses it. Some conserved amino acids are responsible for the difference in the activities of *TFL1* and *FT* (Hanzawa et al. 2005; Ahn et al. 2006). Moreover, amino acids that are essential to the function of TFL1 are conserved in RsTFL1 (Figure 1B). These results therefore indicate that the deduced amino acid sequence of the RsTFL1 protein is quite similar to TFL1, suggesting that *RsTFL1* functions in the same manner as *TFL1*. Genomic DNA gel blot hybridization analysis was performed with a *RsTFL1* cDNA probe, and a single hybridizing restriction fragment was detected (Figure 2), indicating that the radish genome contains only a single copy of *RsTFL1*.

We determined the expression pattern of *RsTFL1* in various radish tissues (Figure 3). A single 376-bp product was amplified from total RNA by using gene-specific primers. RT-PCR analysis revealed that *RsTFL1* was not expressed in dry seeds or imbibed seeds before germination, but was expressed when the cotyledons emerged, 48 h after the initiation of imbibition. *RsTFL1* expression was detected at the shoot apex in both the vegetative and reproductive phases. *TFL1* is expressed in both vegetative and reproductive meristems and regulates floral transition in *Arabidopsis* (Bradley et al. 1997). In contrast, *CENTRORADIALIS* (*CEN*), a *TFL1* ortholog found in *Antirrhinum*, is expressed in reproductive meristems but not in vegetative meristems, and it does not influence floral transition in *Antirrhinum* (Bradley et al. 1996). *RsTFL1* was expressed in both vegetative and reproductive meristems (Figure 3A). This suggests that it may control floral transition in radish. *RsTFL1* transcripts were not detected in leaves or young floral buds, but they were detected in inflorescence stems and floral buds immediately before flowering; moreover, low levels of expression were also detected in pedicels (Figure 3B). *RsTFL1* was expressed in carpels but not in sepals, petals, and stamens. To gain an understanding of the expression pattern of *RsTFL1* during seed maturation, we extracted RNA from developing seeds. *RsTFL1* was expressed 20 d after flowering and continued to be expressed up to 60 d (Figure 4).

In situ hybridization analysis revealed that *RsTFL1* is expressed in the inflorescence meristem but not in the floral meristem (Figure 5A), like in the case of *TFL1* expression in *Arabidopsis*. In vitro transcribed, digoxigenin-labeled antisense and sense RNA probes

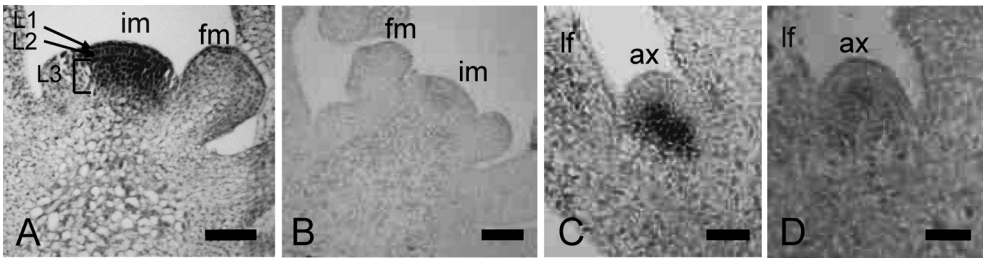

Figure 5. *In situ* localization of *RsTFL1* transcripts in radish. (A) The expression pattern throughout primary inflorescence meristem. (C) The expression pattern in young axillary meristem before floral meristem emergency. (B) and (D) Sections hybridized with sense probe. Plant tissues were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned to 8 μm and affixed to slides at 42°C overnight. RNA *in situ* hybridization, washing under high stringency and detection were carried out as described in the manufacture's protocol (Roche). im, primary inflorescence meristem; fm, floral meristem; ax, axillary meristem; lf, leaf; L1 epidermal layers; L2, subepidermal layers; L3, inner layers. Bar = 100 μm

were synthesized for *RsTFL1*. The apical meristem consists of epidermal (L1), subepidermal (L2), and inner (L3) layers. Although the activity of *TFL1* throughout the apical meristem is necessary to control inflorescence meristem identity, the expression is restricted to the inner cells (part of L3) of apical meristems (Simon et al. 1996; Bradley et al. 1997; Ratcliffe et al. 1999); however, TFL1 protein is present in a wide region of the apical meristem because of its movement to outer cells (L1 and L2). TFL1 functions as a mobile signal across the meristem tissues in order to control the inflorescence meristem (Conti and Bradley 2007). On the other hand, *RsTFL1* was found to be expressed in L1, L2, and a part of L3—within the central region of the inflorescence meristem—unlike the expression pattern of *TFL1* in *Arabidopsis* (Figure 5A). This region is almost coincident with the region in which the TFL1 protein is localized in *Arabidopsis* to control inflorescence identity (Conti and Bradley 2007). This indicates that the RsTFL1 protein is localized solely in the central region and may control indeterminate inflorescence in the absence of a mobile signal. *RsTFL1* expression was restricted to the sub-apical region of the young axillary meristem (Figure 5C). After the development of the floral meristem from the axillary shoot meristem, *RsTFL1* was widely expressed throughout the axillary inflorescence meristem as well as the primary inflorescence meristem (Figure 5A). *FT*, *LFY*, and *TFL1* have antagonistic functions in *Arabidopsis*. FD, a bZIP transcription factor of *Arabidopsis*, was identified as a partner of FT (Abe et al. 2005). Ahn et al. (2006) suggested that FT and TFL1 compete for a common interacting partner (FD) in the shoot apical meristem (SAM). The ratio of *LFY*/*TFL1* activity in the SAM controls the developmental fate of the meristem (Ratcliffe et al. 1999; Ferrandiz et al. 2000). In the *Arabidopsis* SAM, *TFL1* suppresses the floral signals induced by genes that promote flowering, namely, *LFY* and *FT*. Similarly, in radish, *RsTFL1* must also suppress flowering signals in the SAM. However, the mechanism of floral suppression in radish and *Arabidopsis* may differ. Because *RsTFL1* mRNA is present in not only the inner layers but also the outer layers of the inflorescence meristem, it may not always be necessary for the RsTFL1 protein to function as a mobile signal by moving across cells in order to control the inflorescence meristem, like the *Arabidopsis* TFL1 protein. On the basis of the *RsTFL1* expression pattern, we can assume that the RsTFL1 protein localizes quickly throughout the inflorescence meristem, as compared to TFL1. As a result, the radish inflorescence meristem may be able to resist flowering signals, irrespective of the strength of the flowering signals or the intensity of the break-out signal induced by certain environmental factors. Further experiments are needed to elucidate the biological function of *RsTFL1* in radish.

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