TAWAWA1, a regulator of rice inflorescence architecture, functions through the suppression of meristem phase transition

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Inflorsecence structures result from the activities of meristems, which coordinate both the renewal of stem cells in the center and organ formation at the periphery. The fate of a meristem is specified at its initiation and changes as the plant develops. During rice inflorescence development, newly formed meristems acquire a branch meristem (BM) identity, and can generate further meristems or terminate as spikelets. Thus, the form of rice inflorescence is determined by a reiterative pattern of decisions made at the meristems. In the dominant gain-of-function mutant tawawa1-D, the activity of the inflorescence meristem (IM) is extended and spikelet specification is delayed, resulting in prolonged branch formation and increased numbers of spikelets. In contrast, reductions in tawawa1 (TAW1) activity cause precocious IM abortion and spikelet formation, resulting in the generation of small inflorescences. TAW1 encodes a nuclear protein of unknown function and shows high levels of expression in the shoot apical meristem, the IM, and the BMs. TAW1 expression disappears from incipient spikelet meristems (SMs). We also demonstrate that members of the SHORT VEGETATIVE PHASE subfamily of MADS-box genes function downstream of TAW1. We thus propose that TAW1 is a unique regulator of meristem activity in rice and regulates inflorescence development through the promotion of IM activity and suppression of the phase change to SM identity.

A LOG family | meristem identity | grain yield

The timing of each meristem phase change is crucial in the control of inflorescence architecture (1–3). In grass species, the basic architecture of inflorescence is defined by the spatial arrangement of spikelets, which are small branches containing a variable number of flowers. Among the meristems that are generated from the primary inflorescence meristem (IM) during inflorescence (panicle) development, early ones acquire an indeterminate branch meristem (BM) identity, whereas later ones are specified as determinate spikelet meristems (SMs) (Fig. 1A) (4, 5). The BMs themselves are eventually transformed into SMs after generating a certain number of branches and spikelets (Fig. 1B). Thus, the pattern of SM specification is a primary determinant of grass inflorescence form. Delays in SM specification lead to iterations of branching, resulting in larger panicles that could potentially produce more grain. Conversely, the acceleration of SM specification results in smaller panicles with fewer spikelets. Rice inflorescence development exhibits an additional unique feature in that the IM loses its activity after producing several BMs, leaving a vestige at the tip of the rachis, the inflorescence main stem (Fig. 1D). Therefore, the timing of IM abortion is also a critical factor determining the form of rice inflorescence.

The competence of a meristem to become an SM gradually increases during inflorescence development; however, the molecular basis for the timing of SM specification is largely unknown.

To date, several genes that control inflorescence form through the control of meristem phase change have been reported in grass species including rice and maize. Three maize genes, RAMOSA1 (RA1) and RA2 (encoding transcription factors) and RA3 (encoding a trehalose-6-phosphate phosphatase), are positive regulators of SM determination and the shift from long-branch initiation to short-branch initiation (6–9). The mechanisms by which these RA1 genes exert their functions are not well-understood; however, RA1 interacts with a transcriptional corepressor. Orthologs of RA2 and RA3 (but not RA1) are found in the rice genome, but their functions are unknown. The rice genes ABERRANT PANICLE1 (APO1) and APO2 are orthologs of the Arabidopsis genes UNUSUAL FLORAL ORGANS (UFO) and LEAFY (LFY), respectively, and they negatively regulate the shift to SM identity (10–12). In Arabidopsis and other eudicot species, UFO and LFY promote determinate floral meristem identity (10). Thus, APO1 and APO2, which negatively regulate the shift toward determinacy, play opposing roles to those of UFO and LFY. The SM is found specifically in grass species and is an intermediate phase between the indeterminate BM and the floral meristem. This suggests that the genetic framework of inflorescence development may have diverged between the eudicots and the grasses. APO1 was also identified as a major quantitative trait locus (QTL) controlling grain number in the rice panicle, suggesting a tight link between inflorescence branching pattern and yield (13). QTL analyses of genes controlling grain number also identified DENSE PANICLE1, GRAIN NUMBER1, and OsSPL14 as regulators of inflorescence branching. However, these traits have not been considered from the perspective of meristem activity and identity (14–17). To gain a better understanding of the molecular basis of meristem phase change, we analyzed mutations of the TAWAWA1 (TAW1) gene, and propose TAW1 as a unique regulator of SM phase transition.

Results and Discussion
tawawa1-D Plants Form Inflorescences Exhibiting an Increased Branching Phenotype. To understand the molecular links between the control of meristem phase transition and inflorescence structure, we...
identified mutants with altered inflorescence branching patterns. Two lines with increased branching phenotypes were isolated from a screening population in which nDart1, an endogenous rice transposon, actively transposes (Fig. 1C) (18). The mutation was inherited in a semidominant manner in both lines, and subsequent analyses revealed mutations in the same gene.
Fig. 2. Isolation of the TAW1 gene. (A) Insertion of nDart1-0 into a region on chromosome 4 cosegregated with the inflorescence phenotype. (B) Expression levels of genes surrounding the insertion sites in immature inflorescences. Values represent the expression level in heterozygous (taw1-D1/+fl) and homozygous (taw1-D1) mutants relative to that of wild-type plants. (C) Inflorescences in taw1-D1 and its WT plants T65 (Taichung 65). (D) Number of primary branches per inflorescence. n = 7, 5, 9, and 7 for WT (T65), taw1-D1, WT (N), and taw1-4, respectively. Asterisks indicate a significant difference from corresponding control samples (Student’s t test, **P < 0.01). (E) Inflorescences in RNAi and WT plants.

TAW1 was named TAWAWAI (TAW1), from a traditional Japanese word meaning “very fruitful.” taw1-D1 exhibits more severe defects than those of taw1-D2. Both mutant lines show normal growth patterns during the vegetative phase, for example, meristem size, date of leaf initiation, and number of leaves produced. However, in taw1-D1 homozygous plants, stem elongation is suppressed after the transition to reproductive growth, and the inflorescence does not emerge from the leaves (Fig. S1). In both mutant lines, the number of lateral meristems produced on each primary branch is comparable to that of wild-type plants; however, a higher percentage of lateral meristems grows as secondary branches (Fig. 1D). Reiteration of this pattern results in the production of tertiary branches, which are not formed on wild-type plants (Fig. 1E).

In homozygous taw1-D1 mutants (carrying the more severe allele), the increase in inflorescence branching is so extreme that the inflorescence forms an agglomerate with a massive number of undifferentiated meristems (Fig. 1F and G). Such aggregated meristems are also frequently observed in the inflorescences of taw1-D1 heterozygous and taw1-D2 homozygous plants (Fig. 1 H and I). Observations using a scanning electron microscope confirmed that these structures are formed from the repeated production of undifferentiated meristems (Fig. S2). The meristems in taw1-D1 homozygous inflorescences were analyzed for expression of two SM marker genes, FRIZZY PANICLE (FZP) and LEAFY HULL STERILE1 (LHS1)/OsMADS1 (19–21), by in situ hybridization. The lack of expression of these genes indicated that the meristems in the homozygous mutants do not acquire SM identity (Fig. 1 J–M).

TAW1 Encodes a Nuclear Protein Belonging to the ALOG Family. The TAW1 gene was isolated by transposon tagging. An nDart1 insertion (nDart1-0) showed complete cosegregation with the mutant phenotype and was identified by transposon display analysis using segregating siblings of taw1-D1+/fl (Fig. 2A). The transposon was inserted into chromosome 10, between Os10g0477800 and Os10g0478000. Interestingly, the insertion sites in taw1-D1 and taw1-D2 were only 16 bp apart (Fig. S3A). Rough mapping also narrowed the TAW1 locus to this region. Furthermore, revertants obtained from taw1-D1 homozygotes (which became taw1-D1+/fl) and from taw1-D1/+fl (which reverted to wild-type) were accompanied by excisions of nDart1-0 (Fig. S3 B and C). These results indicated that the taw1-D defects were caused by the nDart1-0 insertions in the region between Os10g0477800 and Os10g0478000. Expression of Os10g0478000 was enhanced in the mutant inflorescences, whereas Os10g0477800 and Os10g0478100 were expressed at similar levels in the inflorescences of mutant and wild-type plants (Fig. 2B). Introduction of the genomic region containing Os10g0478000 into wild-type rice plants resulted in a weak taw1-D–like mutant phenotype (Fig. S4). Based on these results, we concluded that Os10g0478000 is the TAW1 gene.

TAW1 encodes a protein of 205 amino acids belonging to the ALOG family, which is named after its earliest identified members (Arabidopsis LSH1 and Oryza G1) (22, 23). The rice genome contains 10 ALOG genes, whereas Arabidopsis has 11 members...
(Fig. S5 A and B) (22–26). To obtain a better understanding of TAW1 function, we obtained two independent loss-of-function mutants, \textit{taw1-3} and \textit{taw1-4}, from a TILLING population. These mutants each carry a missense mutation at the same position (Fig. S5A). \textit{taw1-4} mutants have no obvious defects; however, \textit{taw1-3} mutants produce small inflorescences containing reduced numbers of primary branches (Fig. 2 C and D). A reduction in \textit{TAW1} expression by RNAi resulted in a similar but stronger small inflorescence phenotype in which both primary and secondary branches were reduced (Fig. 2E). In the developing rice inflorescence, the IM, which is derived from the shoot apical meristem (SAM), eventually aborts after producing several primary branches, and is left as a vestige at the base of the topmost primary branch (Fig. 1A). Thus, the timing of IM abortion is a principal determinant for the total number of primary branches in the rice inflorescence. It is likely that the phenotypes in these loss-of-function and knockdown mutants result from the early onset of IM abortion and the precocious specification of SM identity. These results again suggest that \textit{TAW1} regulates the extent of inflorescence branching through the maintenance of IM activity and suppression of the transition to SM identity. Enhanced \textit{TAW1} activity results in a prolonged branching phase, whereas reduced \textit{TAW1} activity accelerates IM degeneration and the acquisition of SM identity, giving rise to an abbreviated branching phase.

**TAW1 Is Expressed in the Meristem to Suppress the Transition to SM Identity.** \textit{TAW1} contains a conserved domain and a nuclear localization signal (Fig. S5A). Indeed, \textit{TAW1} localizes to the nucleus and shows slight but significant activity as a transcriptional activator (Fig. 3 A and B and Fig. S6). We examined the spatiotemporal pattern of \textit{TAW1} mRNA accumulation by in situ hybridization. During the vegetative phase of development, \textit{TAW1} was predominantly expressed in meristems, including the SAM, axillary meristems, and young leaves (Fig. 3 A and C). After transition to the reproductive phase, \textit{TAW1} mRNA continued to accumulate in the IM (Fig. 3D). The strongest signal was observed in BMs in the growing inflorescence (Fig. 3E). After initiation of the primary BMs, \textit{TAW1} expression gradually disappeared from the IM as it degenerated (Fig. 3F). In wild-type inflorescences, the signal intensity in the meristem gradually decreased and became undetectable at SM initiation (Fig. 3G). On the other hand, in \textit{taw1-D} inflorescences, stronger \textit{TAW1} signals were detected, even at the stage when the SMs were formed in the wild-type plants (Fig. 3 H and I). Quantitative PCR analysis indicated that the levels of \textit{TAW1} expression in the mutant inflorescences roughly coincided with the severity of their phenotypes (Fig. 3J). These results imply that \textit{TAW1} functions to suppress SM identity and that its activity must be below a certain threshold level to allow SM specification. Interestingly, \textit{TAW1} expression is reduced in the vegetative shoot apices, leaves, and roots of \textit{taw1-D} mutants compared with wild-type plants (Fig. S7). These results suggest that the sequence surrounding the \textit{nDart1} insertion sites is necessary for fine-tuning the spatial and quantitative expression pattern of \textit{TAW1}. It may be that positive regulators of SM identity interact with this sequence, which is located downstream of the transcribed region. It is also possible that \textit{nDart1} carries a sequence that functions as a transcriptional enhancer; however, this is unlikely because not all \textit{nDart1} insertions activate transcription. Unraveling the molecular mechanisms that regulate \textit{TAW1} transcription will shed light on the regulatory networks controlling rice inflorescence architecture.

The rice genes \textit{G1} and \textit{TH1} are ALOG family members that are expressed in the lemma, palea, and floral organs and control their identity and development (23, 26). On the other hand, the Arabidopsis ALOG family genes \textit{LSH3/OBO1} and \textit{LSH4} are expressed in shoot organ boundary cells (24, 25). The functions of \textit{LSH3/OBO1} and \textit{LSH4} are unknown; however, they induce the overproliferation of shoot meristems and the formation of extra organs when ectopically expressed (25). A plausible scenario is that at least some of the ALOG family proteins share conserved functions, but it may be that their distinctive expression patterns have led to divergent functions. The establishment of the machinery for meristem-specific expression may be a primary reason for the evolution of \textit{TAW1} as a major regulator of meristem activity and phase transition.

**TAW1 Positively Controls the SVP Family MADS-Box Genes.** We next performed a transcriptome analysis using a microarray containing genome-wide cDNAs to discover genes that function downstream of \textit{TAW1} (Fig. 4A). Among the 32 type I MADS-box genes present in the microarray, three genes (\textit{OsMADS22}, \textit{OsMADS47}, and \textit{OsMADS55}) were extensively up-regulated in \textit{taw1-D} mutants. These three genes belong to the \textit{SHORT VEGETATIVE PHASE (SVP)} subfamily (27–29). In contrast, genes involved in spikelet initiation and spikelet organ development, such as \textit{OsMADS7 (SEP3)}, \textit{OsMADS8 (SEP3)}, \textit{OsMADS16 (AP3)}, \textit{OsMADS4 (PI)}, \textit{OsMADS3 (AG)}, and \textit{OsMADS8 (AG)}, showed significant reductions in expression, in keeping with the prolonged
branching phenotype. Ectopic expression of OsMADS47 in the BM in which TAW1 is expressed was confirmed by in situ hybridization analysis (Fig. 4 B and C). These results, together with the known function of SVP genes as suppressors of flowering and inflorescence development (30–33), suggest that the three SVP genes work downstream of TAW1 to suppress SM identity. Using a two-component induction system, we confirmed that the induction of TAW1 transcription does indeed lead to the activation of the three SVP genes (Fig. 4D). Furthermore, the constitutive overexpression of OsMADS22 and OsMADS55 by the cauliflower mosaic virus (CaMV) 35S promoter caused an increase in inflorescence branching (Fig. 4E). These results support the hypothesis that SVP genes function downstream of TAW1. Interestingly, the ectopic expression of SVP genes in barley and maize also resulted in the severe suppression of SM identity, suggesting that SVP function might be conserved among grasses (34–36). In conclusion, our data indicate that TAW1 suppresses the acquisition of SM identity through the induction of SVP genes, which consequently promote or prolong BM identity.

taw1-D2 Is a Moderate Gain-of-Function Allele That May Be Useful for Increasing Grain Yields. Grain number per panicle is one of the four major determinants of rice yield. To test the usefulness of TAW1 mutations for increasing grain yield, we introgressed the moderate taw1-D2 allele into Koshihikari, which is a leading commercial rice cultivar in Japan (Fig. 5 A and B). Field-grown selfed progeny of fifth backcross (BC$_5$F$_2$)-generation plants showed ~45% increases in grain weight per plant (Fig. 5C). These increases were mainly due to extensive increases in grain number per panicle (Fig. 5D), resulting from increases in the numbers of primary, secondary, and tertiary branches (Fig. S8 A–C). Slight decreases in fertility and grain weight were also observed (Fig. 5E and Fig. S8D). Despite the increase in panicle size, the total plant height was slightly reduced in BC$_5$F$_2$ plants (Fig. S8 E–H). Increases in grain number are often linked to decreases in the numbers of branch shoots, called “tillers,” in rice (16, 17). However, this tradeoff effect was not observed in the taw1-D2 introgression line (Fig. S8J).

Conclusion and Perspectives. We propose that TAW1 regulates the inflorescence architecture of rice through the promotion of IM activity and suppression of the phase change to SM identity. The gradual decrease in TAW1 activity during development in wild-type plants may contribute to the increase in competence for acquiring SM identity. Fine-tuning of the temporal TAW1 expression pattern is critical for normal rice inflorescence development. TAW1 function might have evolved to ensure the production of sufficient numbers of spikelets. Unraveling the molecular function of TAW1 will provide an opportunity for a greater understanding of meristem activity and identity, which is a fundamental issue in plant biology. Furthermore, such knowledge could be exploited to further increase crop yields. In addition, TAW1 is of great interest with respect to the evolution of inflorescence architecture (37). TAW1 function may be essential for the development of compound inflorescences, in which the branching pattern is crucial.

Materials and Methods
Plant Materials. taw1-D2 was introgressed into Koshihikari through successive backcrossing five times. Seeds of the resultant BC$_5$F$_2$ plants and Koshihikari were sown on May 28, 2011. Seedlings were transplanted into a paddy field at the Institute of Plant Science and Resources, Okayama University (34.6°N, 133.8°E) with a spacing of 15 cm interhill and 40 cm interrow on June 22, 2011. Fertilizers were applied at the rate of 5:5:5 kg/10 ha of N:P$_2$O$_5$:K$_2$O as basal dressing. Two replications were performed.

Transposon Display. Transposon display was performed according to ref. 38. Genomic DNA samples isolated from taw1-D1 and taw1-D2 mutant siblings were digested with 4-bp restriction enzymes and ligated with adaptors containing an MspI site. PCR products from two cycles of amplification, using the adaptor primer and Dart5-3b-first and Dart5-1,2,3-second/N, were visualized by electrophoresis. Band cosegregating with the taw1-D phenotype were isolated from the gel and amplified with a set of primers, the adaptor primer and Dart5-1,2,3-second/N. The DNA sequence of the amplified band was determined according to ref. 39.

Real-time RT-PCR. Total RNA was extracted using a Plant RNA Isolation Mini Kit (Agilent). After DNase I treatment, first-strand cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen). PCR was performed with SYBR Green I using a Light Cycler 480 System II (Roche Applied Science). The primer sets used to amplify the transcripts are provided in SI Materials and Methods.

Generation of TAW1 knockdown plants. Details of the construction of the binary vectors are provided in SI Materials and Methods. In situ hybridization. In situ hybridizations were performed as described by Kouchi et al. (40). Microtome sections of 10-μm thickness were applied onto glass slides treated with Vectabond (Vector Laboratories). Details of probes are provided in SI Materials and Methods.

Observation of subcellular localization of TAW1-GFP fluorescence. Details of the construction of the binary vectors are provided in SI Materials and Methods. Construction of CaMV 35S-SVPs. Details of the construction of the binary vectors are provided in SI Materials and Methods.

Construction of the GVG-Induction System. Details of the construction of the vectors are provided in SI Materials and Methods.

Tilling. Loss-of-function mutants were obtained from screening populations mutagenized with N-methyl-N-nitrosourea (41, 42).

Assay of Transcriptional Activity. A modified yeast two-hybrid system was used to analyze TAW1 activity to activate transcription. Details of the vector construction and activity assays are provided in SI Materials and Methods.

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12. Terao T, Nagata K, Morino K, Hirose T (2010) A gene controlling the number of primary rachis branches also controls the vascular bundle formation and hence is responsible to increase the harvest index and grain yield in rice. Theor Appl Genet 120(5):875-893.
The primer sets used to amplify the transcripts were as follows: forward and reverse primers for TAWAWA1 (TAW1), 5'-CTAGTTACCTCACCCTCCTC-3' and 5'-GTAGTTTTGCTTGTAGCCAGAG-3'; for OsMADS22, 5'-CCAACTGCAGAGACTGCAATA-3' and 5'-CGAACAGTCAACGCAGGGA-3'; for OsMADS55, 5'-GAGACGTGAGAATGGGAGTAGA-3' and 5'-CATCACCCTGACACACGA-3'; and for Ubiquitin, 5'-AGAAAAGTGCACTCCCTTACC-3' and 5'-GCATGCCAGCAGTAAAACACG-3'. PCR was performed with SYBR Green I using a Light Cycler 480 System II (Roche Applied Science).

**Generation of TAW1 Knockdown Plants.** A 320-bp fragment of the TAW1 cDNA amplified using the primers 5'-CACCGGCGTCAGTCTACCGAGAAG-3' and 5'-ATTAGATGTCAGTAGCAGAGCGACG-3' was cloned into the pENTR/D-TOPO vector (Invitrogen). The resulting vector was named pENTR-TAW1RNAi. pENTR-TAW1RNAi was inserted into the PANDA vector (1) by the LR recombination reaction (Invitrogen). The resulting pPANDA-TAW1RNAi was introduced into rice cultivar (cv.) Nipponbare via Agrobacterium-mediated transformation. The transcription level of TAW1 in the leaf blades of the flag leaf of T0, the first generation regenerated from transformed calli, plants was determined by real-time PCR using Ubiquitin as a standard. The primers used for the quantification of TAW1 and Ubiquitin transcription are described above.

**Generation of Probes for in Situ Hybridization.** A partial cDNA sequence of TAW1 PCR-amplified and cloned into pENTR/D-TOPO vector (Invitrogen) was used as a template to make a RNA probe. A primer set to amplify TAW1 cDNA was 5'-CACCGGCGTCAGTTACCGAGAAG-3', 5'-ATTAGATGTCAGTAGCAGAGCGACG-3'. The probe for OsMADS47 was synthesized using cDNA (J075171C16) provided by the Rice Genome Resource Center (www.rgrc.dna.affrc.go.jp/index.html). Probes for FRIZZY PANCLE and LEAFY HULL STERILE1/OsMADS1 have been previously described (2, 3). To make the antisense probe, in vivo transcription was performed using the linearized plasmid as a template, with the incorporation of digoxigenin-UTP.

**Observation of Subcellular Localization of TAW1-GFP Fluorescence.** A 4,939-bp fragment of the TAW1 genome containing the 3,144-bp upstream, coding, and 1,181-bp downstream regions of the TAW1 gene was amplified with the primers 5'-ACTGGCGTGTGTTTGTAGTC-3' and 5'-GGCATCCAGTGAGACTGAGTG-3'. The amplified fragment was cloned into an SmaI site of pBluescript (pBS) to generate pTAW1G. Subsequently, the TAW1 coding region was removed from pTAW1G by PCR using pTAW1G as a template and the primers 5'-GCCGAGCGACGGTTGTGGTCAAGATC-3' and 5'-CGTCGATCTGCACAAACCAAACAAAC-3'. The amplified fragment (pBS-TAW1G), containing the TAW1 upstream and downstream regions and pBS, was digested with DpnI. The TAW1G coding region amplified from the rice genome using the primers 5'-CACCATGGAGTTCGTGGCGCACGC-3' and 5'-GGCGGCGGCCGCGGAGAGC-3' was cloned into the pENTR/D-TOPO vector (Invitrogen). The resultant vector was inserted into pGWB6 (4) by the LR recombination reaction (Invitrogen) to produce p35S-TAW1:sGFP. A fragment containing the sGFP and TAW1 coding region was amplified from p35S-TAW1:sGFP using the primers 5'-ATGGTGGCAAGGGCAGGAA-3' and 5'-TCAGCGGGCCGGCGGGGAGGAGG-3'. This fragment was digested with DpnI and cloned into the DpnI-digested pBS-TAW1U1D. The resultant plasmid pBS-TAW1g:sGFP was digested with BamHI and SalI and cloned into the binary vector pPZP2H-lac to produce pPPZ-PATAW1g:sGFP. pPPZ-PATAW1gsGFP was transformed into Agrobacterium tumefaciens (EHA105, ref. 5) and used for rice transformation (cv. Nipponbare). Subcellular localization of GFP fluorescence was observed in the T0 generation of transgenic plants under a confocalmicroscopylaser microscope (FV10-ASW; Olympus).

**Construction of Cauliflower Mosaic Virus 35S-SVPs.** A full-length cDNA of SHORT VEGETATIVE PHASEs (SVPs) containing the whole ORF was amplified using the primer set for OsMADS22, 5'-CACCGGCGCGCGCTTGCGTGC-3' and 5'-GAAAGCCTGCAAAGACGACAT-3'. The resulting pPANDA-TAW1RNAi was introduced into rice cultivar (cv.) Nipponbare via Agrobacterium-mediated transformation. The transcription level of TAW1 in the leaf blades of the flag leaf of T0, the first generation regenerated from transformed calli, plants was determined by real-time PCR using Ubiquitin as a standard. The primers used for the quantification of TAW1 and Ubiquitin transcription are described above.

**Construction of the GVG-Induction System.** To make p4UAS::TAW1, the coding region of TAW1 was digested by restriction enzymes and cloned into the SpeI and Xhol sites of pINDEX2 (6). The resulting p4UAS::TAW1 was introduced into calluses derived from mature seeds of rice (cv. Nipponbare) via Agrobacterium-mediated transformation. The base of the leaf blade in T0-generation transgenic plants was used for induction. Cut leaf blades were cultured in water for 1 h and then transferred to Murashige and Skoog liquid medium containing 100 μM dexamethasone (DEX). RNA was isolated after 24 h of DEX treatment.

**Assay of Transcriptional Activity.** A modified yeast two-hybrid (Y2H) system was used to analyze TAW1 activity to activate transcription. Y2H was performed using MATCHMAKER GAL4 Two-Hybrid System 3 (Clontech). The β-galactosidase assay was performed according to the manufacturer’s procedure. The full-length cDNA of TAW1, G1, or LAX, a positive control of an active transcription factor (7), was amplified by PCR, fused to the GAL4-DNA–binding domain (GAL4BD), and cloned into pGBK7 (Clontech). The resultant plasmids, pGBK7-TAW1, pGBK7-G1, and pGBK7-LAX, were transformed into yeast strain Y187 containing the GAL4-UAS-β-galactosidase reporter gene. The activity of TAW1, G1, or LAX to activate transcription of the β-galactosidase gene was measured. β-Galactosidase activity in yeast extracts is indicated.


**Fig. S1.** Stem length in taw1-D mutants. The length of the internode in the uppermost (First), second-uppermost (Second), and third (Third)-uppermost nodes are shown. All data are shown as means ± SD; n = 10 for wild-type, taw1-D1/+, and taw1-D1; n = 20 for taw1-D2/+ and taw1-D2. Asterisks indicate a significant difference from the control (WT) (Student’s t test; *P < 0.05, **P < 0.01).

**Fig. S2.** SEM analysis of a young inflorescence at the spikelet developmental stage. (A) The inflorescence of wild-type plants. Spikelet organs are growing. l, lemma; p, palea; s, stamen. (B and C) The inflorescence of taw1-D1 plants. A large number of undifferentiated meristems are observed (B). A close-up view of the boxed region in B is shown in C. (Scale bars, 200 μm.)

**Fig. S3.** Insertion and excision of nDart1. (A) Insertion sites of nDart1-0 in taw1-D1 and taw1-D2 are shown. Sequences in target site duplication in taw1-D1 and taw1-D2 are indicated in blue and green letters, respectively. (B) A revertant inflorescence (arrowhead). The inflorescence containing spikelets developed in taw1-D1 homozygous plants, from which inflorescences do not emerge from leaves due to the suppression of stem elongation. Spikelets are not produced in taw1-D1 homozygous plants. Thus, it is likely that the inflorescence with spikelets developed from cells containing a somatic reversion caused by an excision of the inserted nDart1. The white box shows the inflorescence. The left image shows the enlarged view of the inflorescence. (Scale bar, 10 cm.) (C) Sequencing analysis of the TAW1 gene in the revertants revealed the excision of nDart1 and an insertion of an extra sequence of 6 or 8 bp in independent reversion events.
**Fig. S4.** Introduction of the TAW1 genome region into WT rice. The TAW1 genome region containing a 3-kb promoter region, coding region, and 1-kb 3′ UTR was introduced into WT rice. Transgenic plants [Gain of TAW1 copy number (GTAW1)] showed the increased branching phenotype, mimicking taw1-D mutants. (A) Inflorescence of WT and the transgenic plant. (Scale bars, 5 cm.) (B) Number of lateral meristems that grow as secondary branches and lateral spikelets on a primary branch. (C) Number of lateral meristems that grow as tertiary branches and lateral spikelets on each secondary branch. (D) Total number of secondary branches produced per inflorescence. (E) Total number of tertiary branches produced per inflorescence. (F) Total number of spikelets produced per inflorescence. All data are shown as means ± SD; n = 3 plants (#28), 4 plants (WT), and 5 plants (#3 and #4). Asterisks indicate a significant difference from the control (WT) (Student’s t test; *P < 0.05, **P < 0.01).
Fig. S5. Analysis of TAW1 protein. (A) Amino acid sequence of TAW1. A conserved region and a nuclear localization signal are indicated with orange and green under the lines, respectively. (B) Phylogenetic tree of ALOG (Arabidopsis LSH1 and Oryza G1) family proteins in rice and Arabidopsis. Genes shown with numbers starting with Os, long sterile lemma (G1), and TRIANGULAR HULL1 (TH1) are rice genes, whereas LIGHT-DEPENDENT-SHORT-HYPOCOTYL (LSH1–LSH10) are Arabidopsis genes. Amino acids in the conserved region were used for the phylogenetic analysis. OBO1, ORGAN BOUNDARY1.

Fig. S6. Activity of TAW1 as a transcriptional activator. β-Galactosidase activities are shown as means of three replications ± SD. G1 (Os07g04670) and LAX1 (Os01g0831000) are an ALOG family protein and a bHLH protein, respectively. Asterisks indicate a significant difference from the control (GAL4BD) (Student’s t test; **P < 0.01).
Fig. S7. Expression pattern of TAW1 in taw1-D mutants. Quantitative RT-PCR analysis of TAW1 expression in the shoot apices, leaves, and roots. The values represent mean TAW1 expression relative to UBIQUITIN ± SD (n = 3).

Fig. S8. Agronomic traits of the selfed progeny of fifth backcross (BC$_5$F$_2$) line. (A) Number of primary branches per panicle. (B) Number of lateral meristems produced on the primary branch. The blue bottom bar indicates secondary branches and the pink upper bar indicates lateral spikelets. (C) Total number of tertiary branches per panicle. (D) Fertility. (E) Panicle length. (F) Diameter of the panicle rachis. (G) Days to heading. (H) Plant height. (I) Total number of panicles per plant. n = 23 plants for Koshihikari (K) and 24 BC$_5$F$_2$ plants homozygous for taw1-D2. BC$_5$F$_2$. All data are shown as means ± SD. Asterisks indicate a significant difference from corresponding control samples of Koshihikari (K) (Student’s t test; *P < 0.05, **P < 0.01).