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Regulation of Root Elongation by Histone Acetylation in *Arabidopsis*

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Transcriptional repression by histone modification represents a universal mechanism that underlies critical biological processes, such as neurogenesis and hematopoietic differentiation, in animals. In plants, however, the extent to which these regulatory pathways are involved in development and morphogenesis is not well defined. SWP1/LDL1 is a component of a plant corepressor complex that is involved in regulation of flower timing. Here, we report that SWP1 also plays a role in the regulation of root elongation by repressing a root-specific gene *lateral root primordium 1* (*LRP1*) via histone deacetylation. A null mutation in *SWP1* results in hyperacetylation of histones H3 and H4 in *LRP1* chromatin, elevation of *LRP1* expression, and increased root elongation. This effect of *SWP1* knockout on the root phenotype is mimicked by transgenic expression of *LRP1*, which bypasses the SWP1-mediated suppression of the native gene. Thus, SWP1 likely functions as a regulator of developmental events both in the shoot and in the root meristem.

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Introduction

Transcriptional repression by covalent modification of core histones represents one of the universal mechanisms for gene regulation, underlying many central developmental processes, such as neurogenesis and hematopoietic differentiation, in vertebrates.[1] In plants, however, the extent to which these regulatory pathways are involved in development and morphogenesis is not well defined. The best-characterized example of such involvement is the repression of the *Arabidopsis* flower timing regulator *FLC* by histone deacetylation and methylation effected by corepressor complexes containing members of the SWP/LDL/FLD family, which contains four proteins characterized by the presence of SWIRM and polyamine oxidase domains.[2–5] It remains unknown whether these repressors may play a role in other aspects of plant development. Here,

we examined this question in regard to the SWP1/LDL1, which has been shown to act as a repressor of *FLC*[4] and, thus, to participate in regulation of flower timing, but which has no known role in the regulation of root growth and/or development. We identified one of the SWP1 target genes as *lateral root primordium 1* (*LRP1*).[6] *LRP1*, which is one of the 10 members of the *SHI* gene family,[7] is known to be expressed in root primordia of *Arabidopsis*.[6] The biological role of *LRP1*, however, remains enigmatic mainly because its insertional mutant develops no apparent phenotype.[6] Here, we show that SWP1 represses *LRP1* by histone deacetylation of its chromatin, and that relief of this repression, either by insertional mutagenesis of *SWP1* or by transgenic expression of *LRP1*, results in increased root elongation.

Results

SWP1 modulates root elongation

To better understand the potential tissue and organ specificity of SWP1, we examined its native pattern of expression and subcellular localization. To this end, SWP1 was internally tagged with the citrine variant

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Abbreviations used: LRP1, *lateral root primordium 1*; YFP, yellow fluorescent protein; RT, reverse transcription; HDAC, histone deacetylase; ChIP, chromatin immunoprecipitation.

of yellow fluorescent protein (YFP)[8] and expressed in transgenic *Arabidopsis* under the control of its native regulatory elements—an approach known to reproduce faithfully the natural expression of the tagged gene.[9,10] These experiments demonstrated that SWP1 is a nuclear protein that is mainly expressed in meristem-rich shoot apical regions (not shown) and in the roots (Fig. 1a). While the SWP1 expression pattern in the shoot was expected based on its function in the control of flowering,[4] the expression in the roots hinted at a potential new role for SWP1 in root development. Interestingly, SWP1 was not expressed equally throughout the roots; instead, its expression occurred in a gradient-like pattern, with maximal accumulation in the peripheral areas of both lateral and primary roots (Fig. 1a): these regions include meristematic and elongating tissues, suggesting involvement of SWP1 in the control of root elongation. Thus, we examined whether a knockout mutation of SWP1, *swp1-1*[4] develops an altered root-length phenotype. Figure 1c shows that, indeed, the *swp1-1* mutant seedlings exhibited a consistent increase in the length of their roots as compared to the wild-type plants, indicating that SWP1 negatively regulates root growth.

SWP1 represses the *LRP1* gene by histone hypoacetylation

That the loss-of-function mutant of SWP1 developed longer roots suggested that this transcriptional repressor targets a positive modulator of root length. To initially identify such a target gene, we performed a cDNA microarray analysis on mRNA isolated from 2-week-old homozygous *swp1-1* seedlings identified in a number of upregulated genes such as *FLC*[4] and several others. Only one of these genes, *LRP1*, was a known root-specific gene.[6] Interestingly, although *LRP1* has long been known to be expressed mainly in the roots, its effect on root development has remained completely obscure.[6] Furthermore, the known pattern of *LRP1* expression[6] is consistent with its repression by SWP1 because, as illustrated in the diagram in Fig. 1b, *LRP1* is expressed in differentiating cells at the base of root primordium, but not at its peripheral areas containing meristematic cells,[6,11] whereas *SWP1* shows an exactly reversed expression pattern (Fig. 1a and b).

Our microarray analysis indicated that *LRP1* expression in the *swp1-1* line is approximately 4.2 times higher then that in the wild-type plants. This derepression of *LRP1* was confirmed by reverse transcription (RT) PCR (Fig. 2a), which demonstrated a distinct increase in the levels of *LRP1* mRNA in the root tissues of the *swp1-1* plants. Quantification of the *LRP1* expression by real-time PCR indicated a 2.4-fold increase in *swp1-1* mutant roots—in agreement with the microarray data (Fig. 2b). Thus, *LRP1* most likely represents a root-specific target gene repressed by SWP1.

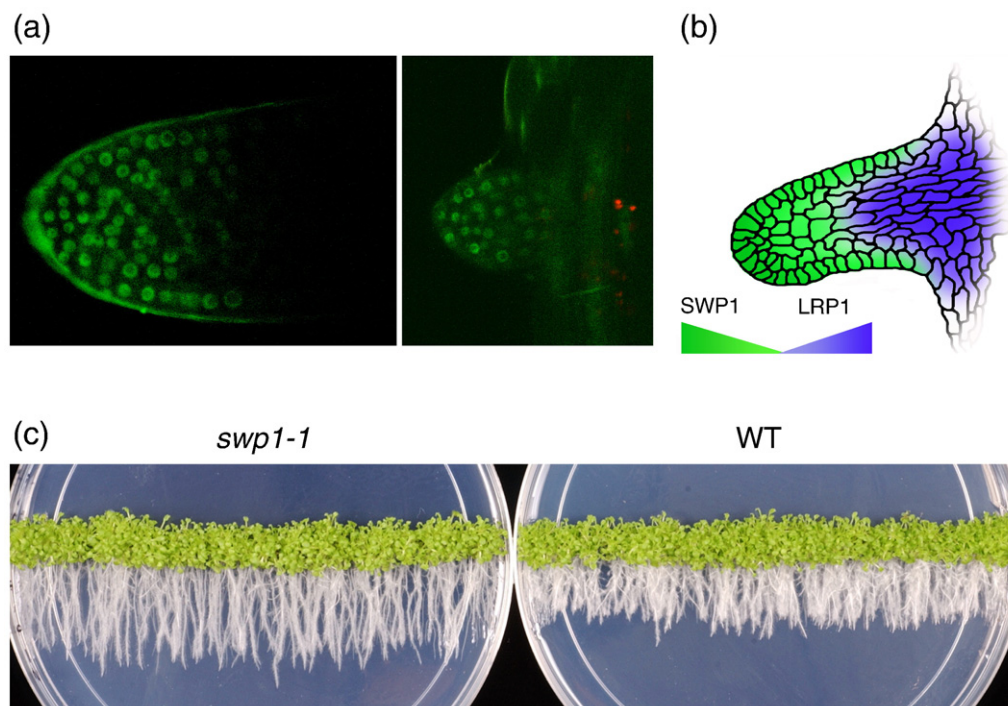Next, we examined the mechanism by which SWP1 may affect *LRP1* gene expression. SWP1 and

Fig. 1. Native pattern of expression and nuclear localization of SWP1 in *Arabidopsis* roots and its effect on root elongation. (a) YFP-tagged SWP1 expressed from its native regulatory elements in primary (left) and emerging (right) lateral roots in 2-week-old seedlings. YFP signal is shown in green, and plastid autofluorescence is shown in red. All images are projections of several confocal sections. (b) Schematic representation of *swp1* (green) and *LRP1* (blue) expressions in root tissue, based on (a) and Smith and Fedoroff,[6] respectively. (c) Loss of SWP1 function leads to increased root elongation in the *swp1-1* mutant as compared to the wild-type (WT) 2-week-old seedlings.
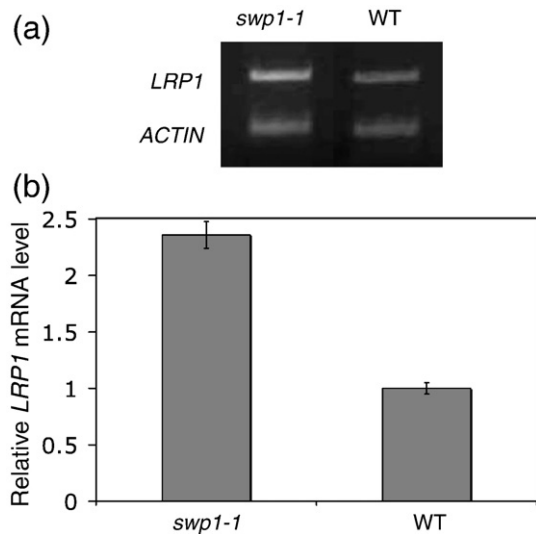
Fig. 2. SWP1 represses *LRP1* gene expression in the *swp1-1* mutant. (a) RT-PCR analysis of *LRP1* derepression. (b) Quantification of *LRP1* derepression by real-time PCR.

its homolog FLD have been proposed to function in a corepressor complex with histone deacetylases (HDACs).[2–5] Thus, we used chromatin immunoprecipitation (ChIP) to examine whether the *SWP1*-mediated repression of *LRP1* occurs via hypoacetylation of the *LRP1* chromatin. Because the regulatory elements of the *LRP1* gene involved in its repression are unknown, we tested several regions within the *LRP1* promoter (Fig. 3a) and within its coding sequence (not shown). These experiments detected statistically significant higher levels of histone H4 and H3 acetylation of the *LRP1* chromatin in the roots of the wild-type plants in comparison to those in the *swp1-1* mutant (Fig. 3b and c). H4 and H3 hyperacetylation, which is diagnostic of active chromatin,[12] was observed in the region of *LRP1* promoter 1.4–1.6 kb upstream of the transcription initiation point (Fig. 3a, region B), whereas no significant differences in other tested regions of the *LRP1* promoter (Fig. 3) or in the *LRP1* coding sequence (not shown) were detected. Thus, *SWP1* is involved in *LRP1* repression by histone deacetylation, perhaps as a component of an HDAC corepressor complex.

Elevated expression of *LRP1* mimics the elongated root phenotype of the *swp1-1* mutant

The correlation between the root-specific phenotype of the *swp1-1* mutant and derepressed expression of *LRP1* in these plants suggests a role for *LRP1* in the mutant phenotype. We tested this idea directly by elevating *LRP1* expression in the wild-type plants via transgenic expression of this gene. RT-PCR analysis of the root tissues of these *LRP1* transgenic plants demonstrated enhanced levels of the transgene expression as compared to the parental wild-type line (Fig. 4a). The *LRP1* transgenic seedlings were then examined for alterations in their root

length. Figure 4b shows that these plants indeed developed roots that were longer than the roots of the wild-type seedlings and were comparable to the roots of the *swp1-1* plants (see Fig. 1c). Next, we performed time-course measurements of the root length of the seedlings of the wild-type, *swp1-1*, and *LRP1* transgenic plant lines. The resulting data were plotted as differences in root length relative to the wild-type root length at each growth interval. Figure 4c shows that, already within 1–2 days of germination, the roots of both *swp1-1* mutant and *LRP1* transgenic lines became longer than the wild-type roots, and the respective maximal length difference of about 55–45% was observed in 4- to 7-day-old seedlings. At later time periods, the differences became somewhat smaller and then stabilized, such that neither of the plant lines returned to the
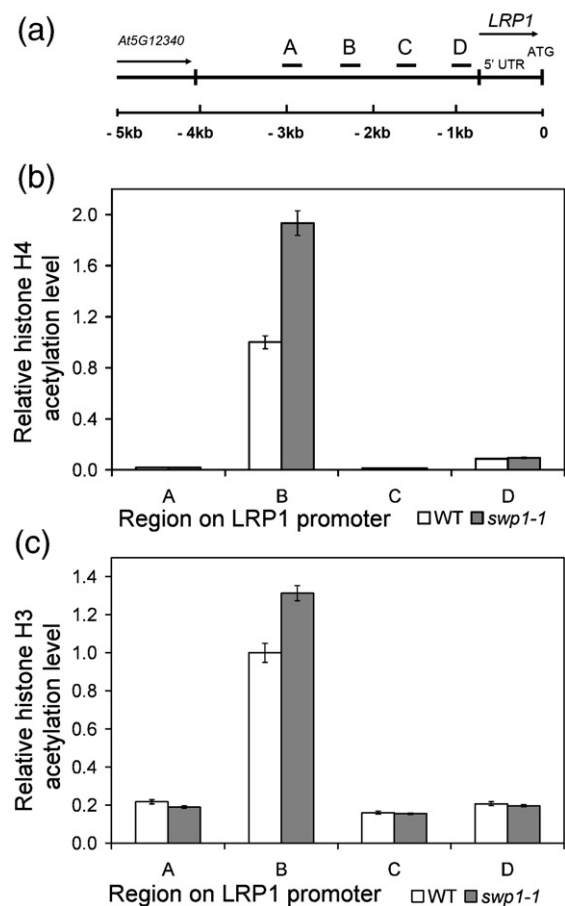

Fig. 3. ChIP analysis of histone acetylation on the *LRP1* promoter in the *swp1-1* and wild-type plants. (a) Schematic representation of the intergenic region between the translation initiation site of *LRP1* and the next upstream gene *At5G12340*. The locations of the *LRP1* promoter regions A–D used for ChIP analyses are indicated. (b) Relative acetylation levels of histone H4. (c) Relative acetylation levels of histone H4. Quantification of acetylation was performed by quantitative PCR following ChIP. Shaded and white boxes indicate the *swp1-1* and wild-type (WT) plants, respectively. At least three independent experiments were conducted for each experimental condition.
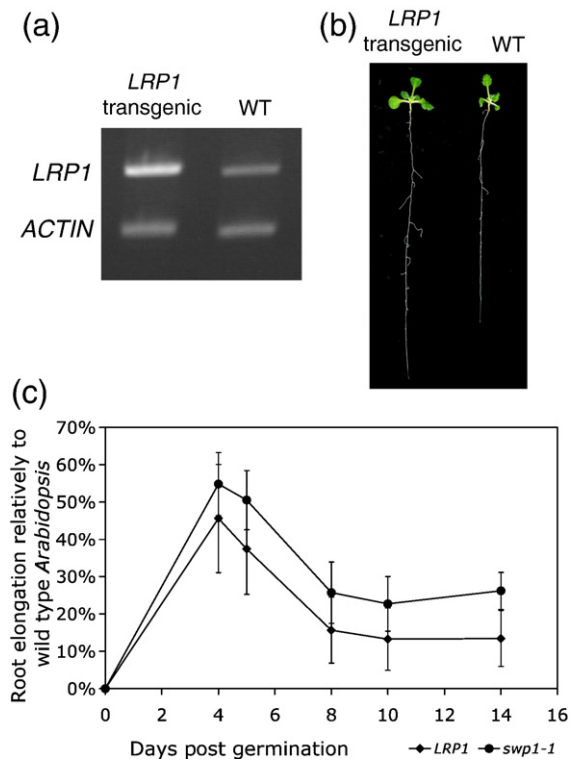
Fig. 4. Increased root elongation in *LRP1* transgenic plants. (a) RT-PCR analysis of *LRP1* expression in the roots of *LRP1* transgenic and wild-type plants. (b) Representative images of roots developed by 2-week-old *LRP1* transgenic and wild-type (WT) seedlings. (c) Time course of root elongation in *LRP1* transgenic (filled diamond) and *swp1-1* (filled circle) seedlings. The data are presented as increase in root length relative to the length of the roots of the wild-type plants at each time point, which was considered 100%. Statistical analysis was based on measurements of the roots of at least 30 seedlings for each time point.

wild-type root lengths for the entire 2-week duration of this experiment (Fig. 4c). Unpaired Student's *t* test confirmed that the root length between *LRP1* transgenic plants and wild-type plants, as well as that between *swp1-1* mutant plants and wild-type plants, differed statistically significantly with *t* values of 2.48 and 5.57, respectively. No other detectable phenotypic alterations, including flower timing, were observed in the *LRP1* transgenic plants. Collectively, these data suggest that *LRP1* transgenic plants phenocopy the *swp1-1* mutation in regard to its effect on root elongation.

Discussion

The involvement of histone acetylation in the regulation of plant root development is just beginning to emerge, with the best-known example of the effects of global histone acetylation on cell patterning observed in root epidermis.[13] We identified a regulatory mechanism in which histone acetylation modulates root elongation. This process is mediated by a transcriptional repressor SWP1 that negatively regulates expression of a root-specific *LRP1* gene via H3 and H4 deacetylation within a defined region of the *LRP1* promoter. Reversal of the SWP1-mediated *LRP1* repression, by inactivation of the *SWP1* gene or by transgenic expression of *LRP1* itself, produced an elongated root phenotype. The extent of this change in root length was consistent but relatively modest, suggesting that the *SWP1*–*LRP1* interaction represents a mechanism for the fine-tuning of root elongation. *LRP1* repression by SWP1 most likely represents the basis for the reported transient and localized pattern of *LRP1* expression[6] and explains why further inactivation of *LRP1* by mutagenesis failed to elicit detectable phenotypes,[6] whereas transgenic expression of *LRP1* produces a clear phenotype. Obviously, the effects of altered *LRP1* expression may also be modulated by the action of other genes of the *SHI* family, which, despite sequence variability, are often functionally redundant and synergistic in their effects on *Arabidopsis* organ development.[7] While the exact mechanism of the *LRP1* activity is unknown, that this protein contains a conserved RING-finger-like zinc finger motif[7] suggests its function as a transcription factor.

SWP1 repressor activity involves histone deacetylation of the *LRP1* chromatin, indicating that SWP1 functions in a repressor complex that should include HDAC(s) and a putative DNA-binding protein to direct it to the *LRP1* promoter. These SWP1-based repressor complexes are likely active mainly within the plant meristem. This notion is based on three lines of evidence: SWP1 expression associates with apical shoot and root-meristem-rich regions; the regulation of *LRP1* expression by SWP1 most likely takes place within the root meristem; and SWP1 also regulates flower timing[14]—a process presumed to occur in the meristem.[15] Consistent with this idea, the null *swp1-1* mutation had no effect on seed germination,[14] which does not involve apical meristem activity.[16,17,18] Taken together, these observations suggest that the SWP1 transcriptional repressor may represent a component of the system for control of meristematic gene expression.

Materials and Methods

Plants

Wild-type, *swp1-1*[14] and *LRP1* transgenic lines were derived from the Columbia ecotype of *Arabidopsis thaliana*. Plants were grown on Gamborg's B5 (Sigma)/0.1% sucrose medium in an environment-controlled chamber at 22–24 °C and maintained under long-day conditions of 16 h of white light (70–80 μmol photons m^{-2} s^{-1}) and 8 h of darkness.

YFP tagging and native expression of *SWP1*

Transgenic *Arabidopsis* plants expressing YFP-tagged full-length SWP1 from its native regulatory sequences

were generated using fluorescent tagging of full-length proteins.[10] First, *SWP1* was amplified from genomic DNA as two fragments using two sets of primers, P1/P2 and P3/P4 (P1: 5′-gctcgatccacctaggctccttcttctgagacttttttattgtgc-3′; P2: 5′-cacagctccacctccacctccaggccggccgatcatcgattcccttcttg-3′; P3: 5′-tgctggtgctgctgcggccgctggggcctcttcactcaaagctgcaagact-3′; P4: 5′-cgtagcgagaccacaggatcggttcttacgggttttcg-3′). For the second PCR, a pair of gene-nonspecific GATEWAY[19] primers (forward: 5′-ggggacaagtttgtacaaaaaagcaggctgctcgatccacctaggct-3′; reverse: 5′-ggggaccactttgtacaagaaagctgggtcgtagcgagaccacagga-3′) was combined with three templates (the YFP sequence derived from pRSET$_B$-Citrine[8] and two *SWP1* fragments), and triple-template PCR was performed to produce the full-length *SWP1* gene, with the YFP sequence inserted 30 bp upstream of the STOP codon.[10] All PCRs were performed using the ExTaq DNA polymerase (TaKaRa, Japan).[10] The resulting triple-template PCR product was recombined into the GATEWAY donor vector pDONR207 (Invitrogen), verified by DNA sequencing, and recombined into the binary destination vector pBIN-GW.[10] This construct was then introduced into the *Agrobacterium tumefaciens* strain GV3101 and used to transform *Arabidopsis* plants as described.[20] Seven kanamycin-resistant T$_1$ transformants were selected for analysis of the YFP-tagged SWP1 expression.

Microscopy

Plant tissue samples were mounted in water, and images were collected with a Zeiss LSM 5 Pascal laser scanning confocal microscope as previously described.[10]

LRP1 transgenic lines

LRP1 cDNA was subcloned into a binary pRCS2-*nptII* vector.[21] *Agrobacterium*-mediated transformation of *Arabidopsis* plants with the *LRP1* expression construct and selection of the transformants were performed as described for the SWP1-YFP plants.

RT-PCR and quantitative real-time PCR analysis

For RT-PCR, total root RNA from 2-week-old seedlings was isolated using TRI reagent (Molecular Research Center) and treated with RNase-free DNase (DNA-free kit; Ambion). cDNA was synthesized using ProtoScript First Strand cDNA Synthesis Kit (New England Biolabs) utilizing oligo-dT primers and approximately 500 ng of the DNA-free RNA for each sample. Equal amounts of the RT reaction products were PCR-amplified using *Actin*-specific (forward: 5′-accttgctggtcgtgacctt-3′; reverse: 5′-gatcccgtcatggaaacgat-3′) and *LRP1*-specific (forward: 5′-atgggcatggttggtctaagagatg-3′; reverse: 5′-actgtaaaacccaccgcctgaacc-3′) primers. Both primer pairs were designed to amplify across introns in order to discriminate between cDNA and potential residual genomic DNA contaminants. The absence of such contamination was also demonstrated by control PCRs performed without RT.

Quantitative real-time PCR was performed using the abovedescribed cDNA preparations in an ABI PRISM 7700 Sequence Detector with iQ SYBR-Green Supermix (Bio-Rad) and the following sets of primers for the *LRP1* and *ACTIN* genes, respectively: 5′-acacaccacctcaaagtttcg-3′ (forward) and 5′-tgccctggccatgctt-3′ (reverse), and 5′-ctcctgctatgtatgttgccattcaagctgttc-3′ (forward) and 5′-gcgtaacctcgtagattggtaccgtgt-3′ (reverse). The relative abundance of the *LRP1* mRNA-specific product was normalized to the amount of the *Actin* mRNA-specific product.

Microarray analysis

The experiments were performed at the Genomic Informatics Center of the University of Rochester Medical School (Rochester, NY) as previously described.[14] Briefly, total RNA was purified from 2-week-old wild-type and mutant seedlings and reverse-transcribed, followed by addition of an initiation site for T7 RNA polymerase at the 3′-end and generation of cRNA. Samples were analyzed using the Affymetrix *Arabidopsis* ATH1 Genome array that queried 24,000 genes. Iobion's GeneTraffic MULTI was used to perform Robust Multi-Chip Analysis, which is a median polishing algorithm used in conjunction with both background subtraction and quantile normalization approaches. Data were analyzed by Statistical Analysis of Microarrays (http://www.fgc.urmc.rochester.edu).

Chromatin immunoprecipitation

Two-week-old seedlings were analyzed by ChIP as previously described[14,22] using anti-acetyl-histone H3 (cat no. 06-599) and anti-acetyl-histone H4 (cat no. 06-598) antibodies (Upstate Biotech). PCR was performed using a LightCycler 480 Real-Time PCR System (Roche) and the following forward and reverse primer pairs corresponding to areas on the *LRP1* promoter: (a) 5′-ttatgcagtttcaagaggaagagtg-3′ and 5′-catatatattgattcccgtctgatc-3′; (b) 5′-ggaaaagaatagtcagaatatgtag-3′ and 5′-ttagagatcgagttccacaagctac-3′; (c) 5′-tagtaaaattggttactactaaacc-3′ and 5′-tggcacacgttgatatctatcttgg-3; and (d) 5′-acgcgtatctaccgaaccatgtacc-3′ and 5′-atggcacatgataccttcttctcc-3′. Relative amounts of PCR products were normalized to the amount of the *Actin*-specific product.[22]

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