Brief Communication

Rice homolog of Sin3-associated polypeptide 30, OsSFL1, mediates histone deacetylation to regulate flowering time during short days

Yuke Geng†, Pingxian Zhang†, Qing Liu, Ziwei Wei, Adeel Riaz, Sadaruddin Chachar and Xiaofeng Gu*

Biotechnology Research Institute, Chinese Academy of Agricultural Science, Beijing, China

Received 25 June 2019; revised 31 July 2019; accepted 5 August 2019.
*Correspondence (Tel +86-010-82105326; fax +86-010-82105326; email guxiaofeng@caas.cn)
†These authors contributed equally to this work.

Keywords: histone deacetylation, heading date, photoperiodic flowering, HDAC, OsSFL1, rice.

The developmental transition from vegetative to reproductive phase (i.e. flowering) is the critical event in plant’s life cycle, which is regulated by exogenous and endogenous signals to ensure it in a timely manner (He and Li, 2018). Rice (Oryza sativa) is classified as a facultative short-day plant (Tsuij et al., 2011). In rice, Hd1 (Heading date 1) is controlled by photoperiod, encodes a zinc finger protein and promotes floral transition under SDs by up-regulating Hd3a (Heading date 3a) expression; while under LDs, it strongly represses Hd3a expression to restrain floral transition (Sun et al., 2014). Histone acetylation levels are mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs remove acetyl group from histones, leading to a condensed chromatin structure and repressing gene expression, whereas HATs relax chromatin structure and activate gene expression (Liu et al., 2014). As one of best characterized HDAC complexes, the Sin3-HDAC consists of the deacetylase enzyme RPD3, the scaffold Sin3-HDAC and the structural proteins Sin3-Associated Polypeptides 18 and 30 (SAP18 and SAP30; Ahringer, 2000). Remarkably, two SAP30-like proteins in Arabidopsis, AFR1and AFR2 have been reported to outline a detailed mechanism in response to light exposure by which histone deacetylation regulates plant flowering at right time (Gu et al., 2013). However, the molecular function of SAP30 homolog in rice and whether it could be involved in regulation of flowering time remained unknown. Our study investigated the rice homolog of yeast SAP30 (SAP30 Functional Like 1, OsSFL1; LOC_Os04g08450) causing delayed flowering under SDs.

To investigate biological function of OsSFL1, we first produced ossfl1 (CRISPR-Cas9 editing) and ossfl1-T (T-DNA insertion) lines (Figure 1a). The editing line, ossfl1, was identified at the first exon with 28-bp deletion of OsSFL1 in Nip background with the target SG sequence (5’-TCTCCGCCTCGTGACAGATGGG-3’) fused into the engineered CRISPR/Cas vector as reported previously (Feng et al., 2013). The ossfl1-T (PFG_3A-07944) L line was obtained from the RiceGE database (http://signal.salk.edu/cgi-bin/RiceGE) with Dongjin (DJ) background and identified as a knockout line with insertion at the second intron. In ossfl1, we observed a significant delay of almost 2 weeks compared with Nip under SDs (Figure 1b); however, no significant difference was observed under LDs (Figure 1c). Consistently, we also verified that ossfl1-T line delayed flowering time under SDs (about 17 days) compared with that of wild-type DJ plants (Figure 1d), suggesting that OsSFL1 is an important player to regulate flowering time in rice.

We then investigated diurnal expression levels of OsSFL1 under SDs. Exposure to light, OsSFL1 was slightly peaked at midday of SD (zeitgeber time 4, ZT4), and after 2.5 h darkness exposure (ZT12), the transcript levels were increased, but decreased before dawn (Figure 1e). The full-length CDS of OsSFL1 was fused with a GFP tag in the PAN580-GFP vector and then transformed into rice protoplasts of 12-day-old etiolated seedlings; this model confirmed that the in-frame OsSFL1-GFP fusion protein was specific to the nucleus (Figure 1f), indicating that the nuclear protein OsSFL1 displayed diurnal expression pattern.

To determine how OsSFL1 regulates the functions of photoperiodic genes, the diurnal expression of Hd1 and Hd3a was detected under SDs. The results showed that the expression level of Hd1 peaked at ZT16 in Nip but peaked at ZT12 in ossfl1 (Figure 1g). Furthermore, in ossfl1, Hd3a transcripts under SDs was significantly reduced at ZT8 (Figure 1h). We then detected Hd1 expression in ossfl1 under LDs. The diurnal expression of Hd1 under LDs was not significantly different between Nip and ossfl1 (Figure 1i). Concomitantly, our results indicated that OsSFL1 was involved in altering photoperiodic rhythm of Hd1 under SDs but not under LDs, and then repressed Hd3a expression at the end of SDs.

Next, we confirmed that if OsSFL1 could directly interact with other Sin3-HDAC members, OsSAP18 and OsHDACs. We conducted Co-IP experiments to check the corresponding interactions by using a transient expression system. The data showed that anti-FLAG (that recognized OsSAP18:FLAG or OsHDAC2:FLAG) efficiently immunoprecipitated OsSFL1:HA, revealing that the OsSFL1 directly interacted with OsSAP18 and OsHDAC2 (Figure 1j), respectively. Collectively, our results suggested that OsSFL1 strongly interacted with OsSAP18 and OsHDAC2 to form OsSFL1-HDAC complex.

Next, to explore whether mutation of OsSFL1 could influence global H3 acetylation level, we performed Western blot assays using 4-week-old rice seedlings grown under SDs incubated with anti-H3 acetyl (K9 + K14 + K18 + K23 + K27) antibody. After visualizing by enhanced chemiluminescence (ECL) systems, we found that global H3 acetylation level was significantly increased in both ossfl1 and ossfl1-T (Figure 1l). Remarkably, with band intensities quantified by the ImageJ software program, we found an almost twofold increase in global H3 acetylation level compared
OsSFL1 regulates flowering time in rice (327)

Figure 1  Loss-of-function alleles of osssf1 delayed flowering time in rice under SDs. (a) Schematic diagram of OsSFL1 mutant lines. The sgRNA mediating the CRISPR–Cas9 target sites of OsSSF1 was indicated by the dotted line. The T-DNA insertion mutant osssf1-T was indicated with hollow triangle. (b) Phenotype of Nip and osssf1 lines under SDs (9.5 h light/14.5 h dark). Bar = 10 cm. (c) Heading date of Nip and osssf1 lines under SDs and LDs (14.5 h light/9.5 h dark). Bars indicated for standard deviation (s.d.); double asterisks indicated statistically significant differences revealed by two-tailed Student’s t test (**, P < 0.01). (d) Heading date of D1 and osssf1-T lines under SDs. (e) Diurnal expression patterns of OsSSF1 in wild-type Nip at SDs. OsAct1 was used as a reference gene. White and dark bars below the X-axis indicate light and dark periods, respectively. (f) Nuclear localization of the OsSSF1-GFP fusion protein in rice protoplasts. OsSSF1-GFP and mCherry fluorescence were imaged using a laser scanning confocal microscope. The nuclear marker OsMADS3 fused with mCherry was used as a nuclear localization control. Scale bars = 50 µm. (g-h) Diurnal expression patterns of Hd1 (g) and Hd3a (h) under SDs. (i) Diurnal expression pattern of Hd1 under LDs. (j-k) Co-immunoprecipitation of OsSSF1 with OsSAP18 (j) or OsHDAC2 (k) in rice protoplasts. Total protein extracts from rice protoplasts transformed with OsSSF1:HA and OsSAP18:FLAG or OsHDAC2:FLAG, then fractionated through SDS-PAGE gel electrophoresis (input) and immunoprecipitated with anti-FLAG agarose (Sigma, Cat#: A2220). The asterisk indicated appearance of input band. (l) The Western blot results immunoblotted with anti-H3acetyl (K9 + K14 + K18 + K23 + K27) (Abcam, Cat#: ab47915) in OsSSF1 mutants and wild types. Band intensities were quantified by the ImageJ program. (m) Verification of synthesized OsSSF1 polyclonal antibody by Western blot (WB) assay (anti-Actin as a control). (n-o) Anti-OsSSF1 (n) and anti-H3 acetyl at K9 + K14 + K18 + K23 + K27 (o) enrichment at Hd1 loci under SDs. The amounts of immunoprecipitated genomic fragments were measured by real-time quantitative PCR and normalized to OsAct1 as an internal control.

to that of wild types (Figure 1f), suggesting that OsSSF1 could mediate histone deacetylation in rice genome.

We further tested whether OsSSF1 could bind to Hd1 chromatin under SDs to mediate periodic histone deacetylation. Firstly, the synthesized OsSSF1 polyclonal antibody was verified by Western blot analysis using total proteins extracted from Nip and osssf1 seedlings (Figure 1m). The ChIP-qPCR data revealed that OsSSF1 proximally bound to the first exon region of Hd1 under SDs in 4-week-old seedlings of Nip and osssf1 (Figure 1n). We then examined H3 acetylation of Hd1 chromatin using an anti-H3 acetyl (K9 + K14 + K18 + K23 + K27) antibody. The results showed that loss of OsSSF1 function could lead to an increase in the H3 acetylation level of Hd1 in the first exon region (Figure 1o), which is consistent with the binding region of OsSSF1. Together, these results concluded that OsSSF1 mediated periodic histone deacetylation on the rhythmically altered photoperiodic gene Hd1 to specifically dampen the expression of its downstream gene Hd3a under SDs.

Our results collectively uncovered a chromatin mechanism of ‘periodic histone deacetylation’ for a day-length-dependent regulation of flowering time in rice. Previous studies have reported that several clock-regulated genes were involved in the alteration of period length and pattern to regulate flowering time in plants (Millar, 2016). Our results collectively uncovered a chromatin mechanism of ‘periodic histone deacetylation’ for a day-length-dependent regulation of flowering time in rice. Previous studies have reported that several clock-regulated genes were involved in the alteration of period length and pattern to regulate flowering time in plants (Millar, 2016). Our results showed that the peak shift in Hd1 expression to an earlier time point might reduce Hd1 stability in osssf1 under SDs (possibly missing timing relative to factors that stabilize Hd1), and the alteration of diurnal and nocturnal expression pattern of Hd1 suppress Hd3a expression. However, Arabidopsis SAP30 homolog proteins (AFRs) have been found repressing FT expression in response to inductive LDs and af1;af2 led to precocious flowering (Gu et al., 2013), which suggests that SAP30 homologs of Arabidopsis and rice have different regulation of photoperiodic pathway. The difference in regulation of flowering time in both species may occur due to different photoperiodic behaviour as rice is a facultative SD plant while Arabidopsis is a facultative LD plant (Tsuji et al., 2011). Moreover, Hd1 (Arabidopsis CO homolog) promotes floral transition through activating Hd3a (Arabidopsis FT homolog) under SDs and strongly represses floral transition via down-regulating Hd3a under LDs, while CO was only found in promotion of FT in Arabidopsis under LDs (Tamaki et al., 2007). Thus, our results and previous report clearly show the existence of diverged potential molecular mechanism underlying SAP30 homologs in plants.

Acknowledgments

We thank National Natural Science Foundation of China (31671670) and National Transgenic Major Program (2019ZX08010-002) to X.G.

Author contributions

X.G. conceived the study and designed the experiments. Y.G. and P.Z. performed the most experiments. Q.L. and Z.W. performed the expression analysis. A.R. and S.C. contributed to the writing. P.Z. and X.G. wrote the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

Ahiringer, J. (2000) NuRD and SIN3: histone deacetylase complexes in development. Trends Genet. 16, 351–356.

Feng, Z., Zhang, B., Ding, W., Liu, X., Yang, D.L., Wei, P., Cao, F. et al. (2013) Efficient genome editing in plants using a CRISPR/Cas system. Cell Res. 23, 1229–1232.

Gu, X., Wang, Y. and He, Y. (2013) Photoperiodic regulation of flowering time through periodic histone deacetylation of the florigen gene FT. Plant Cell 25, e1001649.

He, Y. and Li, Z. (2018) Epigenetic environmental memories in plants: establishment, maintenance, and reprogramming. Trends Genet. 34, 856–866.

Liu, X., Yang, S., Zhao, M., Luo, M., Yu, C.W., Chen, C.Y., Tai, R. et al. (2014) Transcriptional repression by histone deacetylases in plants. Mol. Plant 7, 764–772.

Millar, A.J. (2016) The intracellular dynamics of circadian clocks reach for the light of ecology and evolution. Annu. Rev. Plant Biol. 67, 595–618.

Sun, C., Chen, D., Fang, J., Wang, P., Deng, X. and Chu, C. (2014) Understanding the genetic and epigenetic architecture in complex network of rice flowering pathways. Protein Cell 5, 889–898.

Tamaki, S., Matsu, S., Hannon, L.W., Yokoi, S. and Shimamoto, K. (2007) Hd3a protein is a mobile flowering signal in rice. Science, 316, 1033–1036.

Tsuji, H., Takao, K.I. and Shimamoto, K. (2011) Regulation of flowering in rice: two florigen genes, a complex gene network, and natural variation. Curr. Opin. Plant Biol. 14, 45–52.

© 2019 The Authors. Plant Biotechnology Journal published by Society for Experimental Biology and The Association of Applied Biologists and John Wiley & Sons Ltd., 18, 325–327