A novel domain of *LONG GRAIN 1* in rice possesses transcriptional activation activity

H. Abe,[*1] R. Morita,[*1] Y. Shirakawa,[*1] Y. Hayashi,[*1] H. Ichida,[*1] and T. Abe[*1]

We previously identified the gene *LONG GRAIN 1* (*LIN1*), which regulates seed development, and a mutation of the gene (*lin1*), which causes long grain size.[1] The *lin1* mutant was obtained from rice (*Oryza sativa* L. ssp. *japonica* cv. Nipponbare) M_2 population derived from imbibed seeds irradiated with argon-ion beams (5 Gy, 95 MeV/nucleon, LET: 286 keV m^{-1}) in the RIKEN RI-beam factory.[1] *LIN1* is predicted to encode the 412 amino acid protein, LIN1, with calculated MW 42,579 and pI 10.8. InterPro search of LIN1 revealed that LIN1 has no known functional domain and/or motif.

To gain more insight into LIN1 functions for seed development, we conducted yeast two-hybrid screening for isolation of LIN1 interaction proteins (LIPs).[2] We obtained several LIPs, and one LIP was MADS1, the transcription factor for floral organ development. During the course of the yeast two-hybrid assay for LIN1 and MADS1 interaction, we noticed that mutant lin1 was more actively bound to MADS1 than full length LIN1 (Figs. 2.3 and 4). As sequence alignments of LIN1 homologs in rice and *Arabidopsis* revealed that LIN1 has 5 short stretch conserved domains (I∼V) (Fig. 1.1), we divided LIN1 into several N- and C-terminal fragments, each containing several conserved domains, and performed yeast two-hybrid assays. Unexpectedly, yeast cells transformed with BD-fusion construct of N-terminal fragment of LIN1-M120 (Fig. 1.3) containing only conserved domain I were grown on a selection plate of SD/-Leu/-Trp/-His/-Ade not only in the presence of AD-MADS1 but also in an AD-empty construct (Figs. 2.6 and 7). On the contrary, the BD-fusion construct of the C-terminal fragment of P121-LIN1 (Fig. 1.4) had no such activity (Fig. 2.8). These results indicate that the N-terminal fragment of LIN1-M120 had transcriptional activation activity for the reporter gene expression in yeast cells.

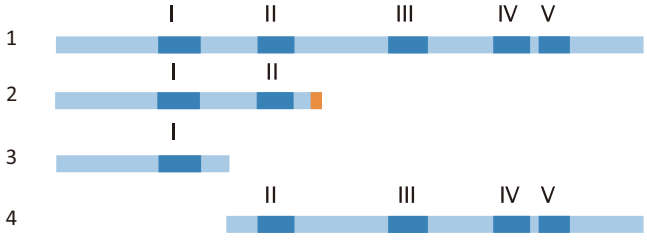

Fig. 1. Schematic diagram of LIN1, lin1 and LIN1 fragment protein sequence. Dark blue color with Roman numerals (I∼V) indicates positions of conserved domains. 1. Full length LIN1 protein (M1-G412). 2. Mutant lin1 protein caused by 1 bp deletion of LIN1 gene. The frame shift mutation result for an extended sequence (orange color) and a premature stop codon. 3. N-terminal fragment of LIN1-M120 containing the domain I. 4. C-terminal fragment of P121-LIN1 without the N-terminal portion (3) of LIN1.

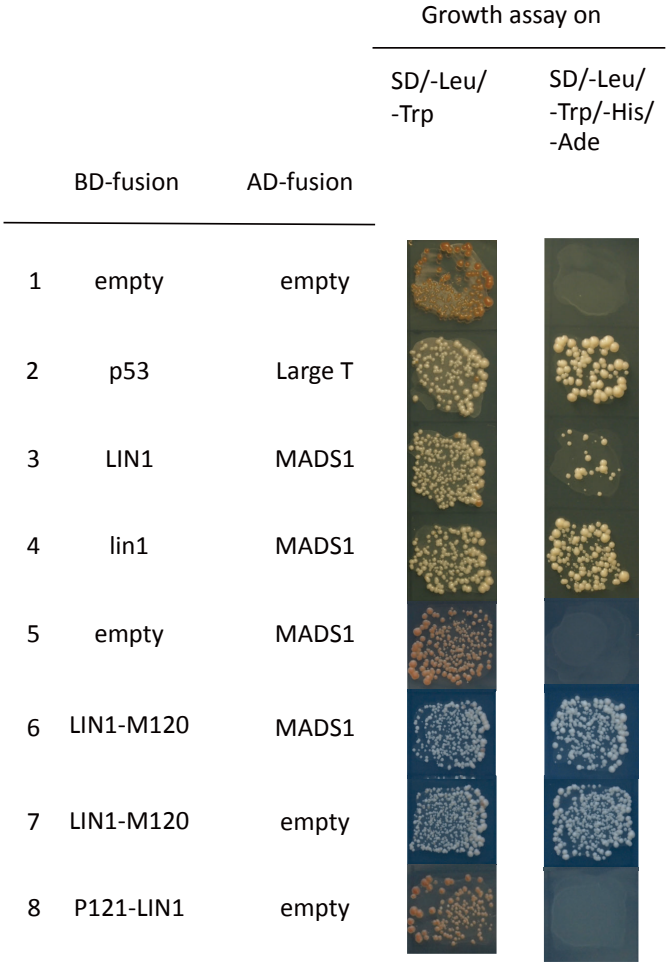

Fig. 2. Yeast two-hybrid assays. Combination of BD-fusion constructs and AD-fusion constructs are indicated (1-8). Both constructs were transformed into Y2H-Gold yeast competent cells, and growth assays on SD/-Leu/-Trp and SD/-Leu/-Trp/-His/-Ade plates were performed.

Considering the results, we hypothesize that LIN1 might be a novel transcription factor family protein with N-terminal transcriptional activation activity for regulating seed development. Elucidation of LIN1-regulated target genes should be provided for important information not only for understanding seed development but also for application of plant breeding.

*1 RIKEN Nishina Center

References

1) R. Morita *et al.*, Mol. Breeding **39**, 135 (2019).
2) H. Abe *et al.*, unpublished.