An early-flowering einkorn wheat mutant with deletions of *PHYTOCLOCK 1/LUX ARRHYTHMO* and *VERNALIZATION 2* exhibits a high level of *VERNALIZATION 1* expression induced by vernalization†

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The early-flowering or early-heading phenotype in bread wheat (*Triticum aestivum*) cultivars is important as it can produce an early harvest. This characteristic is particularly beneficial in East Asia as it allows harvesting to before the onset of the rainy season. To understand the molecular mechanism of flowering in wheat, we developed a large-scale mutant panel in diploid einkorn wheat (*T. monococcum*) using a heavy-ion beam.[1] Einkorn wheat seeds were exposed to a heavy-ion beam and then sown in the field. Selfed seeds from each spike of M_1 plants were used to generate M_2 lines. Every year over the past 15 years, we have obtained approximately 1,000 M_2 lines and built up a mutant panel with 10,000 M_2 lines. This mutant panel is being systematically screened for mutations affecting reproductive growth, especially for the flowering-time mutants. From the large scale mutant panel, we have identified four extra early-flowering mutants, named *extra early-flowering1* (*exe1*), *exe2*, *exe3*, and *exe4*.[2] The four *exe* mutants fall into two groups namely Type I (moderately extra early-flowering type: *exe1* and *exe3*) and Type II (extremely extra early-flowering type: *exe2* and *exe4*). An analysis of *VERNALIZATION 1* (*VRN1*), a flowering promoter gene, shows that it is more highly expressed in seedlings at early developmental stages in both Type I and II mutants than wild-type (WT). These findings indicate that the difference in earliness between Type I and II mutants is associated to the level of *VRN1* expression.

The differences between the diurnal gene expression patterns in the field were examined for four clock-related genes and three clock downstream genes in WT and *exe3* mutant plants grown in the field, respectively. The biggest difference was found for a clock-related gene, *PHYTOCLOCK 1/LUX ARRHYTHMO*, which is abbreviated to *Wheat PCL1* (*WPCL1*). The *WPCL1* was not expressed in the *exe3* mutant plants, whereas it was highly expressed during sunset in WT plants. PCR analysis of DNA markers indicated that the *exe3* mutant had a deletion of *WPCL1* in the genome, which was co-segregated with the mutant phenotype in the segregation line.

We confirmed that the original strain KU104-1 carried a mutation that produced a null allele of a flowering repressor gene *VERNALIZATION 2* (*VRN2*). As a result, the *exe3* mutant has both *WPCL1* and *VRN2* loss-of-function mutations. The analysis of plant development in a growth chamber showed that vernalization treatment accelerated flowering time in the *exe3* mutant under short day (SD) as well as long day (LD) conditions and the early-flowering phenotype was correlated with the earlier up-regulation of *VRN1*. The deletion of *WPCL1* affects the SD-specific expression patterns of some clock-related genes, clock downstream genes, and photoperiod pathway genes, suggesting that the *exe3* mutant causes a disordered SD response. The present study indicates that *VRN1* expression is associated with the biological clock and the *VRN1* up-regulation is not influenced by the presence or absence of *VRN2*.

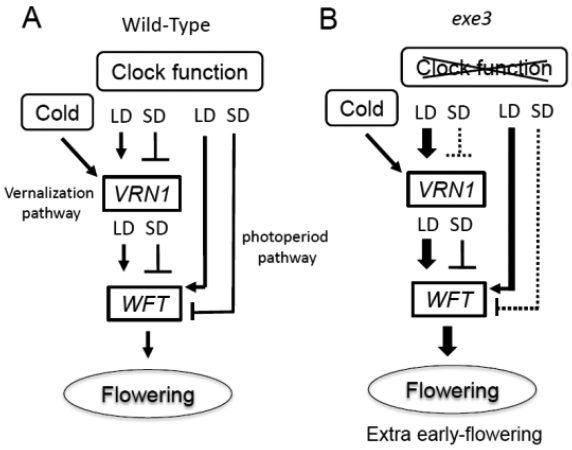

Fig. 1. Model for the extra early-flowering phenotype in the *exe3* mutants compared with wild-type. (A) Wild-type, (B) *exe3* mutant

A model for the extra early-flowering phenotype of *exe3* mutant is shown in Fig. 1. The disruption of clock function also affects the expression of the florigen gene *WFT* through the *VRN1* expression. A high level of *WFT* expression was observed in the *exe3* mutant under LD conditions, suggesting that the disrupted clock somehow induces *WFT* expression. Under SD conditions, another florigen gene could be up-regulated by the disrupted clock function and accelerate flowering in the *exe3* mutant. The up-regulation of *VRN1* is controlled by the vernalization pathway and clock function. It is not related to *VRN2* and determines the earliness in wheat.

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