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SnRK2S, KEY COMPONENTS OF THE OSMOTIC STRESS RESPONSE PATHWAY, INTERACT WITH AND PHOSPHORYLATE FD TRANSCRIPTION FACTOR TO REGULATE FLOWERING TIME

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Osmotic stress, caused by drought or salinity, is a major constraint to agriculture worldwide. Depending on the plant developmental stage in which it is experienced, osmotic stress can inhibit seed germination, reduce plant vegetative growth and affect flowering time. In particular, flowering time is advanced in some species (e.g. wheat) and delayed in others (e.g. rice). SnRK2s protein kinases, are major components of osmotic stress response pathway. In *Arabidopsis thaliana*, SnRK2s family is composed of 10 members (SnRK2.1 to 2.10). With the exception of SnRK2.9, all SnRK2s are activated by osmotic stress treatments, imposed with mannitol or NaCl, but only a subclass, composed of SnRK2.2, 2.3 and 2.6 (and to a lower extent 2.7 and 2.8) is also activated by ABA (Boudsocq et al., 2004 JBC 279**:** 41758–66).

The importance of SnRK2s was recently shown *in vivo* by the extreme osmotic stress sensitivity of a *snrk2.1/2.2/2.3/2.4/2.5/2.6/2.7/2.8/2.9/2.10* decuple mutant (Fujii et al. 2011 PNAS 108:1717-22), while a triple mutant *snrk2.2/2.3/2.6* displayed a nearly abolished sensitivity to ABA (Fujii et al., 2009 PNAS 106:8380-5).

Using a yeast two hybrid screening to unravel new substrates of SnRK2s, we identified FD, a bZIP transcription factor and component of the flowering time integration pathway, as a candidate interactor of SnRK2.4.

Interaction was confirmed using protein pull-down and additional yeast two hybrid experiments were carried out showing that FD interacts with both ABA-dependent and ABA-independent SnRK2s. The region responsible for interaction with SnRK2s was mapped at the N-term of FD protein sequence using deleted fragments of FD.

Using in-gel kinase assays and kinase assays with recombinant proteins produced in *E. coli* we were able to demonstrate that FD is indeed a substrate of endogenous SnRK2s and to map the phosphorylation site within FD protein sequence.

Phenotype analyses of various *snrk2s* mutant combination showed that some mutant combinations display an early flowering phenotype which, accordingly, was associated with a lower expression of direct targets of FD.

The results presented demonstrate a link between flowering time and osmotic stress perception via FD regulation operated by SnRK2s.