Functional analysis of the SWIM domain of Arabidopsis FHY3: seeking evidence for a transposon origin of the FRS gene family

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Introduction

The *Arabidopsis* light-signaling genes FHY3 and FAR1 are the best-studied of the 14 genes composing the FAR1-related sequence (FRS) gene family. They show remarkable homology with MURA, a transposase from the *Mutator*-like element (MULE) gene superfamily (Hudson, *et al.*, 2003; Lin and Wang, 2004). FRS proteins and MULE transposases share significant sequence identity and show striking similarity in their predicted secondary/tertiary structures including an N-terminal C2H2 zinc-chelating motif, a central putative core transposase domain, and a C-terminal SWIM domain (Lin and Wang, 2007). Key aminos such as those at D283 and G305 of FHY3 are conspicuously invariant in the aligned FRS and MURA sequences, and plants carrying the D283N or G305R mutations show an *fhy3* mutant phenotype which can be rescued by a D283N-VP16 or G305R-VP16 fusion protein bearing the herpes simplex virus activation domain, indicating that FHY3 has transcriptional activation activity which has been preserved from an ancestral state (Lin and Wang, 2007).

Taken together, these findings provide good structural evidence for the idea that FRS genes are derived from a relatively ancient domestication event of a Mutator-like transposon. To further support this hypothesis, I will investigate the functional effects on FHY3 activity of mutations at key aminos in the *Arabidopsis* SWIM domain by testing their ability to complement the *fhy3* phenotype when overexpressed *in planta*, the subcellular location of GFP-tagged mutant FHY3, and the effect of mutant FHY3 on downstream gene expression.

Background

The model plant *Arabidopsis thaliana* has several classes of light-sensing proteins, including the red and far-red sensitive phytochromes, the blue-sensitive cryptochromes, and phototropins, which induce plant movement responses to blue light. *Arabidopsis* produces at least five separate phytochromes (PhyA–PhyE). PhyA signals several growth responses, most notably controlling slowed hypocotyl extension and onset of cotyledon expansion when the young seedling emerges from the soil (Wang, 2005).

PhyA exists in two forms. The biologically inactive Pr form is synthesized in the cytosol, from which the light-activated Pfr form is translocated to the nucleus, where it initiates a transcriptional cascade whose details are not yet fully known. Two homologous proteins, FHY3 and FAR1, appear to play a key role in controlling the nuclear translocation process, probably by direct-binding activation of the phyA-interacting proteins FHY1 and FHL. PhyA activation appears to suppress FHY3 and FAR1 suppression, thus forming a "gas and brake" mechanism that ensures the presence of sufficient FHY1 and FHL to enable rapid response to an imminent light signal but shuts down when the signal arrives in order to prevent an exaggerated response (Lin and Wang, 2007).

The ability to maintain this kind of highly-regulated homeostasis is often selectively advantageous. The putative domestication of FRS genes from MULE-like transposases ~110 million years ago would have allowed early plants to control their light responses more effectively and could be a contributing factor in the subsequent landmark success of the angiosperms.

Methods

The Wang lab has approximately 10-12 *fhy3* point mutant *Arabidopsis* lines available, providing an excellent starting point for investigations of both the zinc-finger and SWIM domains. I will focus initially on the activity of SWIM mutants such as C579A and H591A, which are expected to show impaired DNA-binding activity in their C-terminal region. For each transgene, I will begin by testing for light-response phenotypes (e.g. hypocotyl length, cotyledon opening angle) in homozygous seedlings grown under continuous far-red light for five days. FHY3 point mutants overexpressed in an *fhy3* background are expected to show reduced phenotype rescue compared to the wild-type. Many of the transgene constructs have already been tagged with YFP, allowing easy confirmation of any change in subcellular location. The SWIM mutants are not expected to show any change in nuclear localization ability. Downstream target gene expression will be evaluated by RT-PCR. Yeast *LacZ* hybrids will be used as needed to test for domain interaction. The conserved aminos of the SWIM domain are expected to be critical for DNA binding, therefore SWIM mutants are expected to show reduced expression of target genes and reduced interaction with SWIM-specific DNA binding sequences.

References

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