Finding the Missing KASH

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Abstract

The nucleus holds and protects the genetic information of a cell. Nuclear positioning is correlated with several important cellular processes, such as cell division, migration and differentiation, and it is essential to the development of organisms ranging from worms to mammals (Razafsky and Hodzic; Starr and Fridolfsson; Mellad, Warren and Shanahan). Nuclear positioning is mediated through a protein complex across the nuclear envelope (NE), formed by the outer nuclear membrane (ONM) KASH proteins and the inner nuclear membrane (INM) SUN proteins (reviewed in Razafsky and Hodzic; Starr and Fridolfsson; Mellad, Warren and Shanahan). The cytoplasmic part of KASH proteins is associated with the cytoskeleton, and the nucleoplasmic part of SUN proteins is associated with the nucleoskeleton. Meanwhile, in the perinuclear space (PNS), the C-terminal tail of KASH proteins interacts with the SUN domain of SUN proteins. Therefore, KASH-SUN complexes are linkers of the nucleoskeleton to the cytoskeleton (LINC). LINC is conserved from yeasts to humans (Starr and Fridolfsson). However, in plants, only SUN proteins have been identified, leaving plant KASH proteins and the functions of plant SUN proteins a mystery (Graumann, Runions and Evans; Moriguchi et al.;

Murphy, Simmons and Bass). The purpose of this study is to identify plant KASH proteins and uncover the function of the plant KASH-SUN interaction (Zhou et al.).

Results and Discussion

Identification of Plant KASH Proteins. The plant genome does not encode any protein homologous to known KASH proteins. Known KASH proteins have the following features (1) they are ONM tail-anchored proteins, (2) they terminate in a conserved short C-terminal PNS tail, and (3) this PNS tail interacts with the SUN domain of SUN proteins (Razafsky and Hodzic; Starr and Fridolfsson; Mellad, Warren and Shanahan). Plant WPP-domain interacting proteins (WIPs) fulfill the first two features (Xu, Meulia and Meier), which makes WIPs good candidates for plant KASH proteins.

To examine whether the third feature is true for WIPs, a co-immunoprecipitation assay was carried out on Arabidopsis WIPs (AtWIP1, AtWIP2, and AtWIP3) and SUN proteins (AtSUN1 and AtSUN2). RFP-Myc-AtSUN2 was co-expressed with GFP-AtWIP1, GFP-AtWIP1ΔVVPT, and GFP-AtWIP1XT (the PNS tail was mutated, see Fig. 1A for detail) respectively in *Nicotiana benthamiana* leaves. After immunoprecipitation using anti-GFP antibody, the co-immunoprecipitated AtSUN2 was detected by anti-Myc antibody. As shown in Fig. 1B, AtWIP1 interacts with AtSUN2, deleting the last four amino acids reduces the interaction, and mutated the PNS tail diminishes the interaction. Next, the interactions between the other AtWIPs and AtSUN2 were tested using the same technique. The results show that both AtWIP2 and AtWIP3 interact with AtSUN2 (Fig. 1C). Similar experiments were carried out to test the interaction between AtWIPs and AtSUN1, and similar results were obtained (Fig. 1 D and E). Next, AtWIP1 was co-expressed with CFP-AtSUN2, CFP-AtSUN2ΔN (N terminal domain deleted), CFP-AtSUN2ΔCC (coiled-coil domain deleted), CFP-AtSUN2ΔCSUN (C-terminal part of the

SUN domain deleted), and GFP-AtSUN2ΔNSUN (N-terminal part of the SUN domain deleted), respectively. After immunoprecipitated with anti-GFP antibody, co-immunoprecipitated AtWIP1 was detected by anti-AtWIP1 antibody. As shown in Fig. 1G, the CFP-AtSUN2ΔCSUN and GFP-AtSUN2ΔNSUN were unable to bind AtWIP1, indicating that the SUN domain is required for the SUN-WIP interaction. Therefore, the PNS tail of WIPs interacts with the SUN domain of SUN proteins, suggesting that WIPs are plant KASH proteins.

*Function of the WIP-SUN complex***.** It is known that the NE localization of KASH proteins depends on KASH-SUN interactions (Padmakumar et al.; Crisp et al.; Ketema et al.; Stewart-Hutchinson et al.). Therefore, we tested whether the NE localization of AtWIP1 depends on AtSUNs. GFP-AtWIP1 was observed using confocal microscopy, and its NE signal was quantified by the nuclear localization index (NLI), which is the sum of the two maximum NE intensities (N_1+N_2) divided by the sum of the two maximum cytoplasmic (C_1+C_2) intensities (Fig. 2A). Shown by the NLI, the NE localization of GFP-WIP1 was diminished in a *sun1-knockout sun2-knockdown* mutant (*sun1-KO sun2-KD*) when compared with that in wild type (Fig. 2 A and B). Mutating the PNS tail of AtWIP1 led to a similar effect in wild type (Fig. 2 A and B), indicating that the NE localization of WIP1 depends on the WIP-SUN interaction.

Most known KASH-SUN interactions are involved in nuclear positioning, however, no changes of nuclear positioning were observed in either *wip1-1 wip2-1 wip3-1* triple knockout mutant or *sun1-KO sun2-KD*, suggesting that the WIP-SUN complex has a different function. It is known that the cytoplasmic part of WIPs is involved in anchoring Ran GTPase activating enzyme (RanGAP) to the NE (Xu, Meulia and Meier). Observed using confocal microscopy and quantified by NLI, the NE localization of Arabidopsis RanGAP1 (AtRanGAP1) was determined. AtRanGAP1-GFP was diffused from the NE in *sun1-KO sun2-KD*, which is similar to the

AtRanGAP1-GFP signal in *wip1-1 wip2-1 wip3-1* (Fig. 2 C and D), indicating that the WIP-SUN complex functions in AtRanGAP1 NE anchorage. To test whether SUN proteins anchor RanGAP1 to the NE through WIPs, GFP-AtRanGAP1and RFP-Myc-AtSUN2 were co-expressed with either AtWIP1 or AtWIP1XT in *N. benthamiana* leaves. AtRanGAP1-GFP was immunoprecipitated by anti-GFP antibody, and co-immunoprecipitated WIP1 and AtSUN2 were detected as co-immunoprecipitated proteins. Although AtWIP1XT was co-immunoprecipitated, AtSUN2 was not detected as the co-immunoprecipitated protein. This confirms the existence of a RanGAP1-WIP1-SUN2 triple complex and suggests that WIP and SUN are involved in RanGAP1 NE anchorage as shown by the model in Fig. 2F.

Conclusion

In summary, WIPs are plant KASH proteins which interact with SUN proteins at the NE functioning in RanGAP NE anchorage. These discoveries implicates that: (1) KASH-SUN complexes are evolutionarily conserved in plants, but plant KASH proteins have diverged from their non-plant counterparts; (2) The function of RanGAP NE anchorage is novel to KASH-SUN complexes, suggests that they play more roles than nuclear positioning; (3) identifying WIPs as plant KASH proteins reveals the probability of the existence of plant KASH proteins involved in LINC complexes and could shed light on uncovering the mechanism of plant nuclear positioning.

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Figures

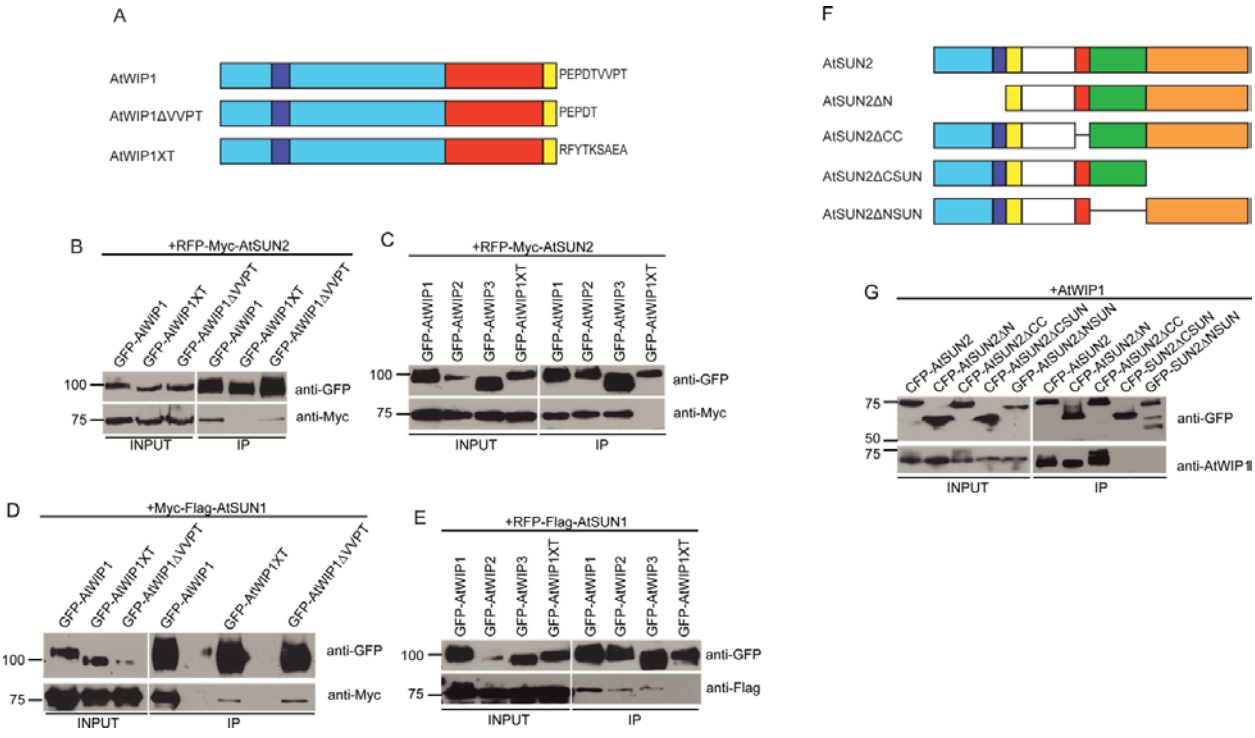

Figure 1 **Characterization of AtSUN-AtWIP interactions.**

(A) Domain organization of AtWIP1 and its mutant derivatives. AtWIP1 consists of an N-terminal domain with unknown function (cyan), a NLS signal (blue), a coiled-coil domain binding AtRanGAP1 (red), a predicted TMD (yellow), and a PNS tail (shown by residue sequences). Figures were drawn to scale.

(B) AtWIP1 interacts with AtSUN2 through its PNS tail.

(C) AtWIP1, AtWIP2, and AtWIP3 interact with AtSUN2.

(D) AtWIP1 interacts with AtSUN1 through its PNS tail.

(E) AtWIP1, AtWIP2, and AtWIP3 interact with AtSUN1.

(F) Domain organization of AtSUN2 and its deletion constructs. AtSUN2 consists of an N-terminal domain with unknown function (cyan), a NLS (blue), a TMD (yellow) followed by a unknown domain (white), a coiled-coil domain (red), and the SUN domain which was split to an N-terminal part (green) and a C-terminal part (orange) in this study. Figures were drawn to scale.

(G) AtSUN2 interacts with AtWIP1 through its SUN domain.

In (B) - (E) and (G), GFP- or CFP-tagged proteins were immunoprecipitated (IP) and detected by anti-GFP antibody. RFP-Myc- or Myc-Flag-tagged proteins were detected by anti-Myc antibody and RFP-Flag tagged proteins were detected by anti-Flag antibody. The input/IP ratio is 1/10. Numbers on the left indicate molecular mass in kilodalton.

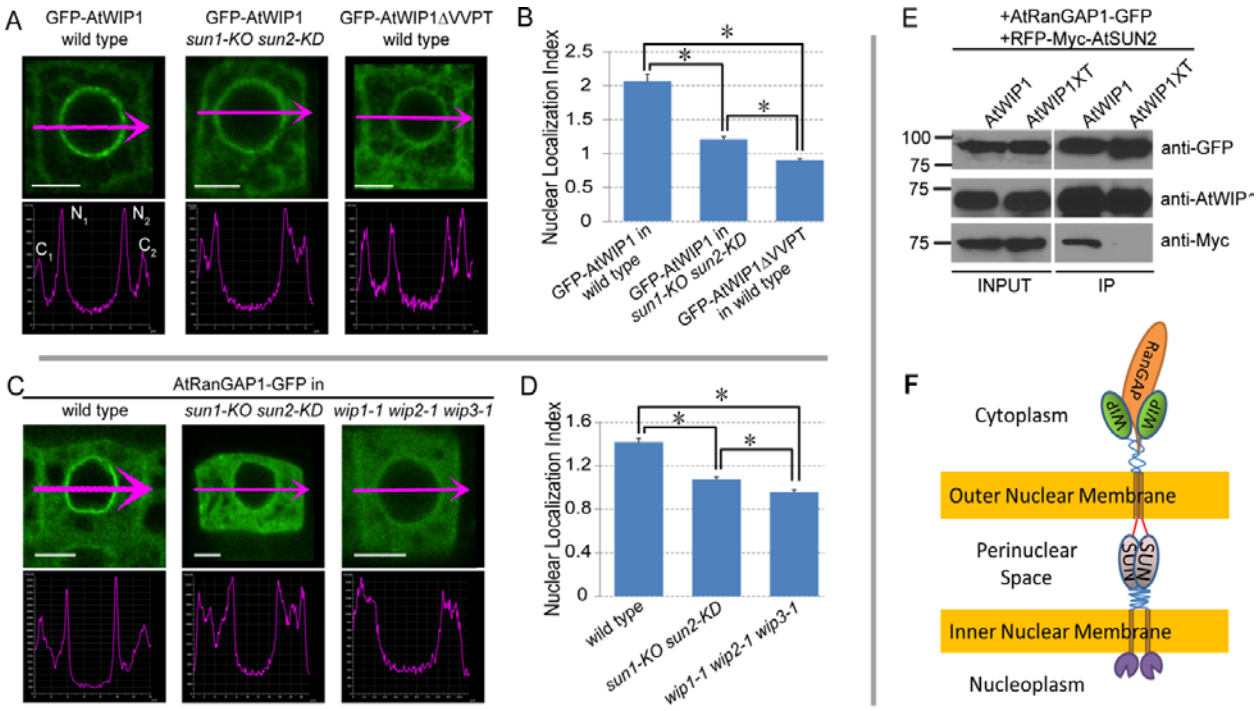

Figure 2

(A) GFP-AtWIP1 or GFP-AtWIP1ΔVVPT signal in undifferentiated root cells (top panel) and corresponding intensity profiles along the magenta arrows (bottom panel). C1, C2, cytoplasmic intensity 1, cytoplasmic intensity 2, respectively; N1, N2, nuclear intensity 1, nuclear intensity 2, respectively.

(B) NE localization index ([N1+N2]/[C1+C2]) calculated using the intensities measured as shown in (A). Asterisks indicate significant statistical difference between compared lines (t-test, $P<0.01$, n=50). Error bars represent standard errors.

(C) AtRanGAP1-GFP signal in undifferentiated root cells (top panel) and corresponding intensity profiles along the magenta arrows (bottom panel).

(D) NE localization index calculated as described in (B), using the intensities measured as shown in (C). Asterisks indicate significant statistical difference between compared lines (t-test, $P<0.01$, n=55). Error bars represent standard errors.

(E) AtSUN2, AtWIP1, and AtRanGAP1 are in the same complex. AtRanGAP1-GFP was immunoprecipitated and detected by anti-GFP antibody. AtWIP1 and AtWIP1XT were detected with anti-WIP1 antibody. RFP-Myc-AtSUN2 was detected with anti-Myc antibody. Numbers on the left indicate molecular mass in kilodalton.

(F) The model of SUN-WIP-RanGAP triple complex at the NE.