The *Arabidopsis* KIN17 and its homolog KLP mediate different aspects of plant growth and development

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Proteins harboring the kIN17 domain (KIN17) constitute a family of well-conserved eukaryotic nuclear proteins involved in nucleic acid metabolism. In mammals, KIN17 orthologs contribute to DNA replication, RNA splicing, and DNA integrity maintenance. Recently, we reported a functional characterization of an *Arabidopsis thaliana* KIN17 homolog (AtKIN17) that uncovered a role for this protein in tuning physiological responses during copper (Cu) deficiency and oxidative stress. However, functions similar to those described in mammals may also be expected in plants given the conservation of functional domains in KIN17 orthologs. Here, we provide additional data consistent with the participation of AtKIN17 in controlling general plant growth and development, as well as in response to UV radiation. Furthermore, the *Arabidopsis* genome codes for a second homolog to KIN17, we referred to as KIN17-LIKE-PROTEIN (KLP). KLP loss-of-function lines exhibited a reduced inhibition of root growth in response to copper excess and relatively elongated hypocotyls in etiolated seedlings. Altogether, our experimental data point to a general function of the kIN17 domain proteins in plant growth and development.

KIN17 represents a family of DNA/RNA-binding proteins conserved in virtually all eukaryotic organisms. All family members encompass a central domain named kIN17 (Pfam PF10357), the exact function of which, however, is not yet well understood. Although its folding largely resembles those of the winged helix DNA-binding domains, a series of structural divergences preclude a direct role in binding to nucleic acids.1 Hence, 2 additional conserved domains in KIN17 proteins confer the DNA/RNA binding ability: a C2H2-like zinc finger (ZF) and a KOW domain.2-4 Several studies in mammals reported that KIN17 participates in replication, RNA processing, and preserving the integrity of DNA following exposure to UV radiation or genotoxic agents.5-7 Recently, we have provided evidence for the physical interaction between *Arabidopsis* AtKIN17 and SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE7 (SPL7), an SBP-domain transcription factor and main orchestrator of the Cu-deficiency response.8-10 In particular, we predicted AtKIN17 to fine-tune SPL7 target activity by promoting SPL7 function when Cu is limiting. Furthermore, AtKIN17 seemed also involved in countering the oxidative stress under Cu scarcity. Altogether, a link between Cu homeostasis, DNA stability, and oxidative stress through the AtKIN17-SPL7 node was proposed.9

In our recent work, we attributed the lack of obvious phenotypic aberrations in the kIN17-1 mutant line to residual AtKIN17 transcripts levels.9 Therefore, we generated transgenics expressing 2 different artificial miRNAs targeting KIN17 transcripts (amiRNA#1 and...
amiRNA lines were still green in the seedling stage (Fig. 1B). This appeared to correlate with a delay in shoot maturation, as amiRNA lines were still green while wild-type plants had already senesced (Fig. 1B). Interestingly, some amiRNA transgenic plants failed to bolt and others gained a bushy appearance due to an early developmental arrest of the main inflorescence followed by precocious proliferation of axillary shoots (Fig. 1B). Moreover, most of the flowers aborted development at some stage before seed maturation and thus seriously compromised fertility of these transgenics (Fig. 1B). Altogether, these results suggest an additional role for AtKIN17 to fulfill in plants by maintaining proper shoot development and proliferation.

Notably, Arabidopsis possesses another KIN17 homolog (hereinafter referred to as KIN17-LIKE-PROTEIN or KLP; At5g51795). Despite a ca. 63% identity with AtKIN17 and the presence of both the k17 and KOW domains, KLP shows 2 main deviations when compared with AtKIN17: 1) a phenylalanine residue (F44) replaces the first cysteine residue (C28) in the AtKIN17 ZF, and 2) PSORT in silico prediction pointed to a preferential cytoplasmic localization, despite the presence of a conserved putative nuclear localization signal (NLS) (Fig. 2A). Indeed, a translational fusion between KLP and the green fluorescent protein (GFP::KLP) corroborated the latter prediction and was found to distribute between cytoplasm and nucleus, whereas GFP::AtKIN17 concentrated in nuclear speckles (Fig. 2B). Thus, given the divergences in domains and subcellular localization, AtKIN17 and KLP are expected to play some different roles in Arabidopsis.

In an attempt to identify KLP-dependent processes, 2 independent mutant lines (klp-1 and klp-2) both carrying a T-DNA insertion in the coding region were investigated (Fig. S2B-C). Gene expression assays revealed that the klp-1 line resulted in a virtual knock-out mutant, whereas the transcript levels of sequences downstream of the T-DNA insertion in klp-2 were raised by ca. 45% compared with wild type in 7-d-old seedlings grown on standard ½ MS (Fig. S2D). Then, klp mutants were submitted to several physiological tests, among them the Cu and etiolation response. With respect to the Cu response, seedlings were grown for 7 d on ½ MS media supplemented with different concentrations of Cu. In agreement with previous reports, ca. 40% inhibition of the wild-type main root growth was monitored in the presence of 50 µM Cu (Fig. 1C; S2A). A similar degree of inhibition could be observed for the k17-1 line, the complemented k17-1, and a double mutant k17-1 klp-1. However, klp single mutants displayed a less dramatic effect with ca. 25% inhibition (Figs. 1C; S2A). This phenotype was
considered Cu-dependent given that only silver ions mimicked the above-described pattern (Fig. S2B). Interestingly, hypocotyls of 3-d etiolated klp seedlings were found to be ca. 25% longer in comparison to identical treated seedlings of wild type, kin17-1 and kin17-1 klp-1 lines (Fig. 1D). Therefore, in the light of the identical behavior displayed by both klp mutants, we first concluded that the T-DNA insertion in klp-2 prevents the formation of a functional KLP protein version despite raised transcript levels. Second, a possible function of KLP in controlling organ elongation may be discerned from mutant root and hypocotyl phenotypes.

Since KIN17 homologs in mammals are reported to participate in maintaining the integrity of DNA when exposed to damaging radiation, we decided to examine their similarity. Indeed, the phenotypic divergences in domains and subcellular localization. Thus, KIN17 and KLP proteins cover different roles in Arabidopsis, despite their similarity. Indeed, the phenotypic alterations described for kin17-1 and klp-1 individual lines are not enhanced in the double mutant. Carlier and collaborators proposed a role for the kin17 domain in maintaining protein-protein interactions, rather than binding to nucleic acid. Accordingly, the functional effects of UV radiation. These roles could be related to either a more general role in preserving DNA integrity and in particular after genotoxic treatments, as described in mammals. Therefore, a reduced protection against DNA damage should also be taken into account in order to explain the dramatic phenotypes previously reported for kin17-1 spl7-2 in front of the oxidative stress following Cu deficiency. Furthermore, KLP, a less well conserved KIN17 family member found in Arabidopsis, seems to exert a repressive activity to modulate growth of certain organs as roots and hypocotyls during Cu excess and darkness, respectively.

Figure 2. AtKIN17 and KLP exhibit divergences in domains and subcellular localization. (A) Protein sequence alignment of AtKIN17 and KLP. AtKIN17 and KLP amino acid sequences were aligned with ClustalW within the MacVector software package. Stars indicate conserved residues, whereas colons (:) and periods (.) mark residues with similar properties. The relative position of the amino acid residues is provided at the right. The conserved domains predicted according to the Conserved Domains interface (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) are highlighted in brown (zinc finger), purple (kin17) and red (KOW). The blue arrow points the substitution of AtKIN17 Cys27 into KLP Phe44. The predicted nuclear localization signals are highlighted in blue. (B) AtKIN17 and KLP subcellular localization. The entire AtKIN17 and KLP coding sequences were N-terminally fused in frame to green fluorescent protein (GFP) and transiently expressed in tobacco leaf epidermal cells. In each case representative confocal microscopy images for the GFP signal and a corresponding bright field image are provided and merged. Scale bars represent 10 μm.
AtK previously proposed role of AtK response mitigates the oxidative stress generated, mostly at the chloroplast. Whenever the protein loss-of-function lines points to a function of K production of to this strategy, the induction of genes participating in the so-called c
Figure 3.

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differences between both Arabidopsis kin17 domain-proteins could be attributable to their respective interactomes. Nonetheless, note that kin17-1 klp-1 behaves like kin17-1 lines, but masks the kpl root and hypocotyl phenotypes. Thus, a genetic interaction between both genes is inferred. If so, KIN17 would be epistatic over KLP. Taken together, our initial experimental data justify the usefulness of a more in-depth study of the role kin17 domain proteins play in plant development.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Supplemental Materials
Supplemental materials may be found here: www.landesbioscience. com/journals/psb/article/28634

Figure 3. An extended working model to integrate the different functions of the 2 Arabidopsis kin17-domain proteins. During Cu deficiency conditions, AtKIN17 physically associates with SPL7 to promote a genetic response aimed at optimizing Cu reallocation and usage. As part of this strategy, the induction of genes participating in the so-called Cu-independent antioxidant response mitigates the oxidative stress generated, mostly at the chloroplast. Whenever the production of ROS exceeds the cellular antioxidant barrier, DNA damage is caused. Besides the previously proposed role of AtKIN17 in the oxidative stress attenuation, a more global function of AtKIN17 in DNA integrity maintenance can be inferred in the light of the response of kin17-1 lines to UV treatments. Therefore, AtKIN17 would be important to overcome the DNA damage upon Cu limitation. However, an initial characterization of the second Arabidopsis kin17 domain protein loss-of-function lines points to a function of KLP to modulate organ growth, e.g., repressing hypocotyl and root growth under etiolation and Cu excess treatments, respectively.