Plant Signaling & Behavior 9, e28634; April; © 2014 Landes Bioscience

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.

Keywords: *Arabidopsis*, Cu response, UV response, abiotic stress, kin17 domain-proteins, plant growth

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Submitted: 03/12/2014

Accepted: 03/24/2014

Published Online: 04/08/2014

Citation: Garcia-Molina A, Xing S, Huijser P. The Arabidopsis KIN17 and its homolog KLP mediate different aspects of plant growth and development. Plant Signaling & Behavior 2014; 9:e28634; PMID: 24713636; http://dx.doi.org/10.4161/psb.28634

Addendum to: Garcia-Molina A, Xing S, Huijser P. A conserved KIN17 curved DNA-binding domain protein assembles with SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE7 to adapt Arabidopsis growth and development to limiting copper availability. Plant Physiol 2014; 164:828–40; PMID:24335506; http://dx.doi.org/10.1104/pp.113.228239

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 counteracting 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

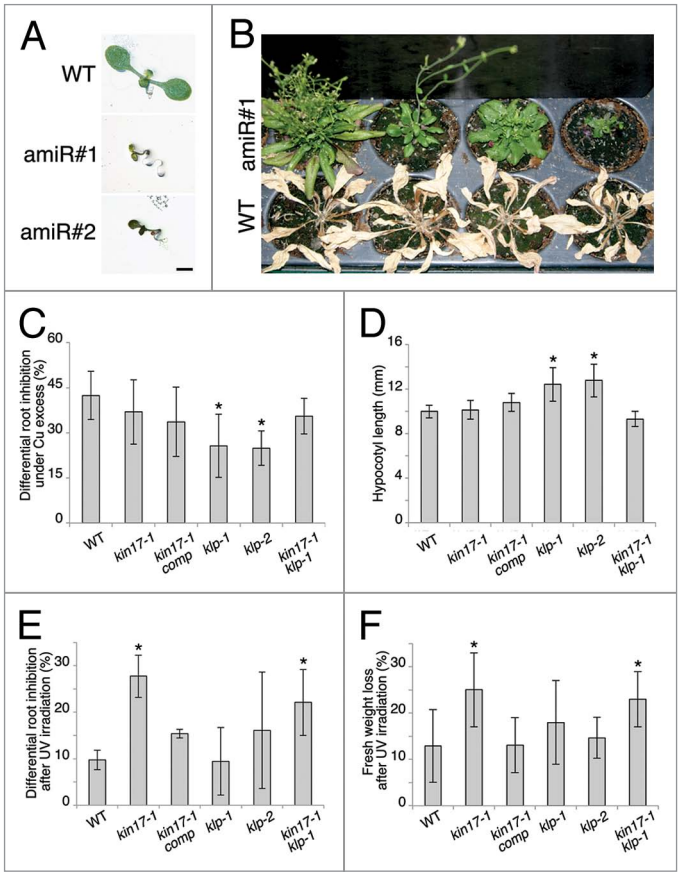

Figure 1. Characteristic phenotypes observed in the *Arabidopsis* kin17 domain proteins loss-of-function lines. (**A**) Representative pictures of different transgenic seedlings expressing the amiRNA#1 (amiR#1) and amiRNA#2 (amiR#2) targeting *AtKIN17* showing arrested or delayed growth in comparison to wild type (WT) when grown on ½ MS medium. Scale bar: 100 µm. (**B**) Pictures of adult wild-type (WT) and amiRNA#1 (amiR#1) transgenic plants grown on standard soils. (**C**) Differential root growth was measured on 7-d-old seedlings corresponding to wild type (WT), *kin17*-1, complemented *kin17*-1, *klp*-1, *klp*-2, and *kin17*-1 *klp*-1 when grown on vertical agar plates containing ½ MS or the same medium supplemented with $CuSO_4$ 50 µM. (**D**) Hypocotyl length of etiolated seedlings from the lines described in (**C**) were determined after germination on wet filter paper and 4 d in darkness. In all panels, the mean of at least 3 independent measurements is provided with error bars corresponding to the standard deviation. Asterisks indicate statistical significant differences to wild type (the Student *t* test, $P < 0.05$). (**E**) Inhibitory root growth assays were performed with 4-d-old seedlings corresponding to the indicated lines grown on vertical agar plates containing ½ MS and irradiated with UV (850 J/m^2). The differential root growth was measured 24 h after irradiation and compared with non-treated seedlings. (**F**) Fresh weight loss in response to UV radiation was determined for the above-mentioned lines after growing horizontally and periodically irradiated (450 J/m^2) every 2 days for 3 times. Fresh weight of 5 seedlings was scored 2 d after the third irradiation.

amiRNA#2) to obtain additional knockdown lines and explore new roles for *AtKIN17* (**Fig. S1A**). The individual and independent transformants showed different degrees of abnormality consisting in strong growth retardation during the seedling stage (**Fig. 1A**). 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 KIN17 and the presence of both the kin17 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 distribution, 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 knockout 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 responses. 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[11,12] 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 *kin17*-1 line, the complemented *kin17*-1, and a double mutant *kin17*-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,[5-7] we decided to evaluate sensitivity to UV of the *kin17*-1, *klp*-1, *klp*-2 single, and *kin17*-1 *klp*-1 double mutants (**Fig S2E**). Root growth inhibitory tests upon UV-irradiation resulted in ca. 15% decreased root length in wild type, complemented *kin17*-1 and both *klp* single mutants in comparison to non-UV irradiated control seedlings, while ca. 25% inhibition was scored in *kin17*-1 and *kin17*-1 *klp*-1 lines (**Fig. 1E**). Similarly, *kin17*-1 and *kin17*-1 *klp*-1 seedlings periodically subjected to UV treatment suffered an overall growth inhibition that was accompanied by a loss of ca. 25% fresh weight in comparison to a ca. 10% loss for the rest of the lines (**Fig. 1F**). Based on these data, we concluded that down-regulation of *KIN17*, but not *KLP*, leads to an enhanced UV sensitivity. This supports the idea that KIN17 participates as a conserved determinant in the protection against UV-radiation damage in both animals and plants.

In conclusion, we report previously unknown functions for *Arabidopsis* kin17 domain-like proteins and extend our previous model to reinforce the proposed interconnection between the Cu deficiency response and DNA metabolism[9] (**Fig. 3**). A more comprehensive analysis of *AtKIN17* loss-of-function lines uncovered the importance of AtKIN17 to maintain plant growth and development and to counteract the negative 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.[5-7] 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.[9] 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. 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[1] proposed a role for the kin17 domain in maintaining protein-protein interactions, rather than binding to nucleic acid. Accordingly, the functional

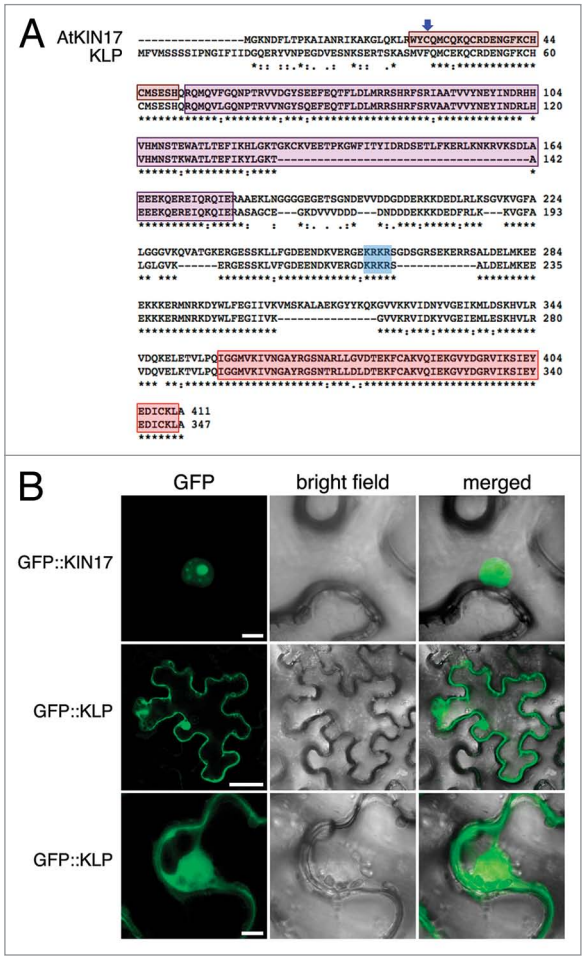

Figure 2. AtKIN17 and KLP exhibit divergences in domains and subcellular localization. (**A**) Protein sequence alignment of AtKIN17 and KLP. AtKIN17 and KLP amino acid sequences were aligned with ClustalW within the MacVector software package. Stars indicate conserved residues, whereas colons (:) and 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.

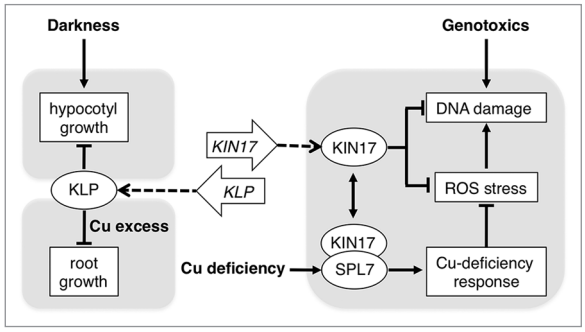

Figure 3. An extended working model to integrate the different functions of the 2 *Arabidopsis* kin17-domain proteins. During Cu deficiency conditions, AtKIN17 physically associates with SPL7 to promote a genetic response aimed at optimizing Cu reallocation and usage. As part of this strategy, the induction 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.

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

References

1. Carlier L, Couprie J, le Maire A, Guilhaudis L, Milazzo-Segalas I, Courçon M, Moutiez M, Gondry M, Davoust D, Gilquin B, et al. Solution structure of the region 51-160 of human KIN17 reveals an atypical winged helix domain. Protein Sci 2007; 16:2750-5; PMID:18029424; http://dx.doi.org/10.1110/ps.073079107
2. Kyrpides NC, Woese CR, Ouzounis CA. KOW: a novel motif linking a bacterial transcription factor with ribosomal proteins. Trends Biochem Sci 1996; 21:425-6; PMID:8987397; http://dx.doi.org/10.1016/S0968-0004(96)30036-4
3. le Maire A, Schiltz M, Stura EA, Pinon-Lataillade G, Couprie J, Moutiez M, Gondry M, Angulo JF, Zinn-Justin S. A tandem of SH3-like domains participates in RNA binding in KIN17, a human protein activated in response to genotoxics. J Mol Biol 2006; 364:764-76; PMID:17045609; http://dx.doi.org/10.1016/j.jmb.2006.09.033
4. Mazin A, Milot E, Devoret R, Chartrand P. KIN17, a mouse nuclear protein, binds to bent DNA fragments that are found at illegitimate recombination junctions in mammalian cells. Mol Gen Genet 1994; 244:435-8; PMID:8078469; http://dx.doi.org/10.1007/BF00286696
5. Biard DS, Miccoli L, Despras E, Frobert Y, Creminon C, Angulo JF. Ionizing radiation triggers chromatin-bound kin17 complex formation in human cells. J Biol Chem 2002; 277:19156-65; PMID:11880372; http://dx.doi.org/10.1074/jbc.M200321200
6. Kannouche P, Pinon-Lataillade G, Tissier A, Chevalier-Lagente O, Sarasin A, Mezzina M, Angulo JF. The nuclear concentration of kin17, a mouse protein that binds to curved DNA, increases during cell proliferation and after UV irradiation. Carcinogenesis 1998; 19:781-9; PMID:9635863; http://dx.doi.org/10.1093/carcin/19.5.781
7. Masson C, Menaa F, Pinon-Lataillade G, Frobert Y, Chevillard S, Radicella JP, Sarasin A, Angulo JF. Global genome repair is required to activate KIN17, a UVC-responsive gene involved in DNA replication. Proc Natl Acad Sci U S A 2003; 100:616-21; PMID:12525703; http://dx.doi.org/10.1073/pnas.0236176100
8. Bernal M, Casero D, Singh V, Wilson GT, Grande A, Yang H, Dodani SC, Pellegrini M, Huijser P, Connolly EL, et al. Transcriptome sequencing identifies SPL7-regulated copper acquisition genes FRO4/FRO5 and the copper dependence of iron homeostasis in Arabidopsis. Plant Cell 2012; 24:738-61; PMID:22374396; http://dx.doi.org/10.1105/tpc.111.090431
9. Garcia-Molina A, Xing S, Huijser P. A conserved KIN17 curved DNA-binding domain protein assembles with SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE7 to adapt Arabidopsis growth and development to limiting copper availability. Plant Physiol 2014; 164:828-40; PMID:24335506; http://dx.doi.org/10.1104/pp.113.228239
10. Yamasaki H, Hayashi M, Fukazawa M, Kobayashi Y, Shikanai T. SQUAMOSA Promoter Binding Protein-Like7 Is a Central Regulator for Copper Homeostasis in Arabidopsis. Plant Cell 2009; 21:347-61; PMID:19122104; http://dx.doi.org/10.1105/tpc.108.060137
11. Andrés-Colás N, Perea-García A, Mayo de Andrés S, Garcia-Molina A, Dorcey E, Rodríguez-Navarro S, Pérez-Amador MA, Puig S, Peñarrubia L. Comparison of global responses to mild deficiency and excess copper levels in Arabidopsis seedlings. Metallomics 2013; 5:1234-46; PMID:23455955; http://dx.doi.org/10.1039/c3mt00025g
12. Lequeux H, Hermans C, Lutts S, Verbruggen N. Response to copper excess in Arabidopsis thaliana: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol Biochem 2010; 48:673-82; PMID:20542443; http://dx.doi.org/10.1016/j.plaphy.2010.05.005