

Communication is key: Reducing DEK1 activity reveals a link between cell-cell contacts and epidermal cell differentiation status

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Abbreviations: DEK1, Defective Kernel 1; HD-ZIP, homeodomain leucine zipper; TF, transcription factor; PC, pavement cell; GC, giant cell; RLK, receptor-like kinase; ACR4, Arabidopsis Crinkly 4.

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Addendum to: "Defective Kernel 1 promotes and maintains plant epidermal differentiation." Roberta Galletti, Kim L. Johnson, Simon Scofield, Rita San-Bento, Andrea M. Watt, James A. H. Murray and Gwyneth C Ingram. doi: 10.1242/dev.122325.

Plant epidermis development requires not only the initial acquisition of tissue identity, but also the ability to differentiate specific cell types over time and to maintain these differentiated states throughout the plant life. To set-up and maintain differentiation, plants activate specific transcriptional programs. Interfering with these programs can prevent differentiation and/or force differentiated cells to lose their identity and re-enter a proliferative state. We have recently shown that the Arabidopsis Defective Kernel 1 (DEK1) protein is required both for the differentiation of epidermal cells and for the maintenance of their fully differentiated state. Defects in DEK1 activity lead to a deregulation of the expression of epidermis-specific differentiation-promoting HD-ZIP IV transcription factors. Here we propose a working model in which DEK1, by maintaining cell-cell contacts, and thus communication between neighboring cells, influences HD-ZIP IV gene expression and epidermis differentiation.

An intact epidermis is critical both for plant growth and plant development. The epidermal layer provides mechanical support for non-lignified tissues, and is also thought to be a key site for the control of growth and morphogenesis.¹

The presence of a continuous epidermal layer is also critically important for defense against pathogens, as well as for coping with abiotic stresses. By secreting highly hydrophobic compounds (cuticular waxes) into the outer cell wall, epidermal cells limit evaporation and the entry of toxic molecules, and establish a physical barrier to the entrance of many microbial

pests.² The differentiation of specialized cell types, such as stomatal guard cells, fine-tunes epidermal function, in particular with regard to the regulation of gas exchange and hydric stress.

The generation of a specific function-adapted tissue such the epidermis, implies a tight growth co-ordination during development and expansion of tissues. For example, in the photosynthetic leaf tissues of Arabidopsis plants, the epidermal layer must concomitantly produce both mature pavement cells (PC), with a full palette of sizes and shapes that form a perfect jigsaw that allows the blade to grow flat (optimization for maximum light capture and structural stabilization), and correctly spaced pairs of stomatal guard cells.³ In the case of true leaves, the temporally and spatially-controlled production of trichomes, has to be included in this complex mosaic. Other organs produce other specialized epidermal cells types. For example in sepal epidermis, differentiated and highly endoreduplicated giant cells (GCs), which are thought to be important for regulating sepal curvature, have to be produced along side smaller cells.⁴

DEK1 is a key protein in epidermis development and differentiation

Previously published work has identified DEFECTIVE KERNEL1 (DEK1) as a regulator of embryo development and growth in plants.⁵⁻⁸ Loss of DEK1 function has been shown to cause early embryo lethality both in Arabidopsis and maize, and a strong down-regulation of DEK1 activity has been shown to lead to a loss of epidermal identity and uncontrolled cell proliferation.^{5,7,9} The DEK1 protein is encoded by a single gene and contains a large number of

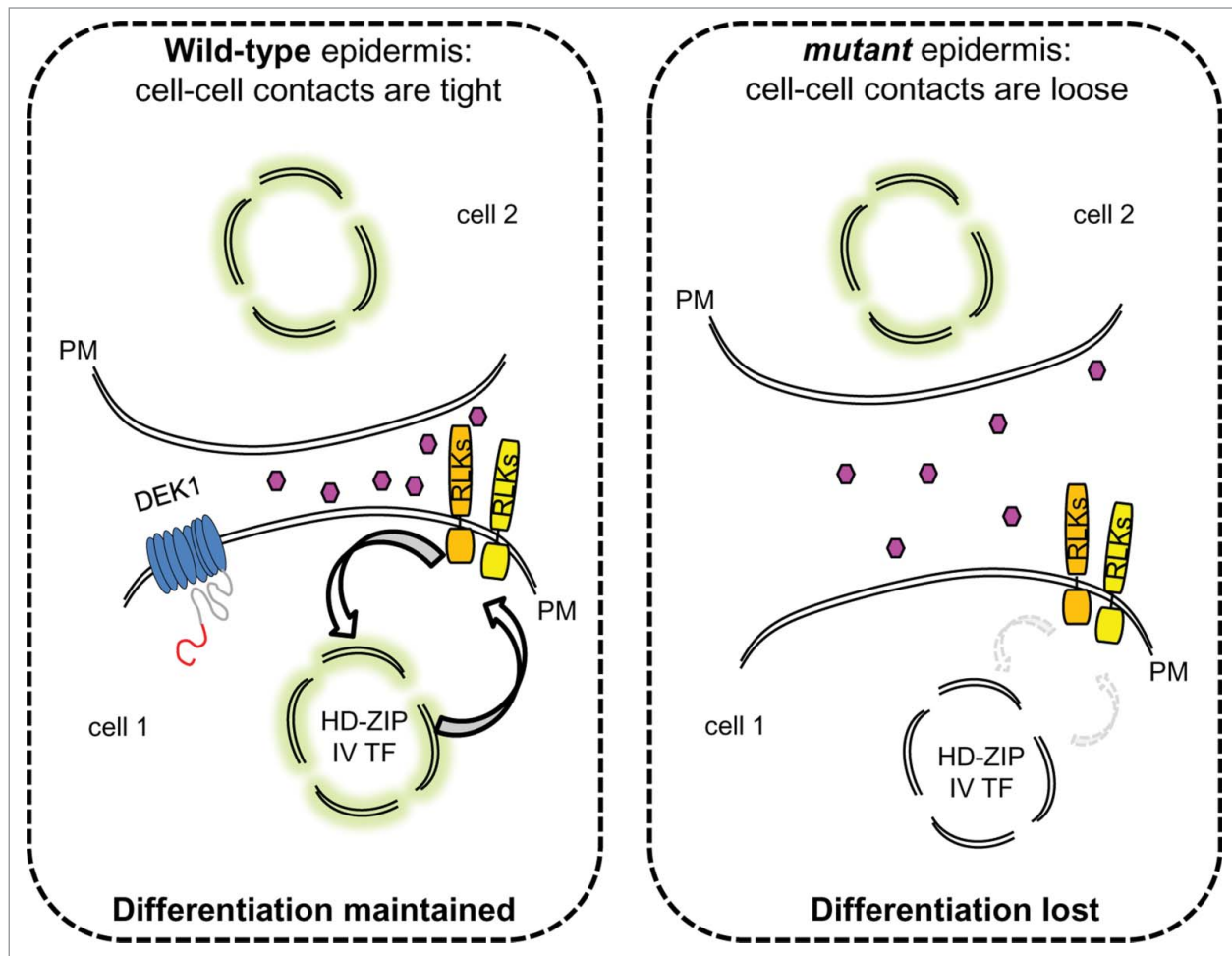


Figure 1. Model explaining DEK1 action on *HD-ZIP IV* gene expression. Purple hexagons represent ligands. DEK1, Defective Kernel 1; HD-ZIP, homeodomain leucine zipper; PM, plasma membranes; RLKs, Receptor-like Kinases.

predicted transmembrane domains (TM) interrupted by a loop, the cellular localization of which is still debated,^{5,10} a cytosolic tail containing a linker domain juxtaposed to the membrane and a C-terminal domain with strong sequence similarity to animal calcium-activated cysteine proteases (calpains).⁸ The calpain domain can be subdivided into a catalytic domain, and a C2-like domain, which likely mediates interactions with membrane compartments.^{8,11} Full length DEK1 protein has been shown to localize in the plasma membrane (PM) of cells, while the free calpain has been reported to be mainly localized in the cytoplasm and/or internal membranes,⁶ and nuclei (personal communication).

Complementation studies have shown that the calpain domain, together with the C2-like domain, is sufficient to complement loss of function *dek1* alleles, suggesting that this is the active domain of the protein,^{6,12} while the TM domains have been proposed to be the regulatory region of the protein, possibly forming a sensor perceiving an extracellular or a plasma membrane-located signal.⁶

By using a weak allele of *DEK1*⁴ and artificial microRNA lines, to finely modulate *DEK1* expression levels, we have recently shown that this protein plays a key role in regulating epidermis differentiation and differentiation maintenance beyond embryogenesis in *Arabidopsis* plants.¹³ Notably, the epidermis of fully expanded cotyledons retains protodermal

characteristics in plants with reduced DEK1 activity, and these phenotypes are not accompanied by alterations in ploidy levels or in the expression of cell cycle-related genes. Moreover, in adult plants, the differentiated state of highly endoreduplicated epidermal structures such as trichomes and GCs, is not correctly maintained when DEK1 activity is reduced, leading to their re-entry into cytokinesis. We have shown that these epidermal defects are coupled with a reduction in the transcript levels of several genes encoding members of the HD-ZIP family IV TFs.¹³ Interestingly, despite the reproducible and apparently co-ordinated decrease in expression of these genes in plants with reduced DEK1 activity, over-expression of the active domain of DEK1, which causes

opposite developmental phenotypes to those observed in weak loss of function alleles,⁶ does not lead to any significant and reproducible increase in the expression of HD-ZIP IV TF transcripts analyzed. We therefore conclude either that regulation of HD-ZIP IV encoding genes by DEK1 is indirect, or that it is complex and requires additional factors.¹³

HD-ZIP family IV TFs have been implicated in several aspects of epidermis development in both the model plant *Arabidopsis*, and commercially relevant species.¹⁴ The *Arabidopsis* HDG2 protein has been shown to be involved in stomatal development,¹⁵ while *AtML1* is involved in GC formation,⁴ and together with PDF2, in epidermis cell fate determination.^{16,17} Likewise HDG11 participates in determining GC identity,⁴ and acts redundantly with HDG12 to regulate trichome branching.¹⁸ Finally, *Glabra2* (*GL2/At-HB 10*) regulates trichome formation and controls the fate of root hairless epidermal cells.¹⁹

Importantly, it has been shown that in *Arabidopsis*, the expression of *atML1* and PDF2 is maintained and stabilized by intracellular signaling mediated by the receptor-like kinase (RLK) *Arabidopsis* Crinkly 4 (*ACR4*).¹⁶ It has also been proposed that apoplastic modifications might affect ligand accumulation and interfere with this feedback loop.²⁰

DEK1 as a regulator of epidermis integrity and cell-cell contacts

It has been shown that strong reduction of DEK1 activity leads to loss of epidermis integrity during embryogenesis,^{6,13} while the weak *DEK1* allele *dek1-4* has epidermal cell junctions which have variable heights, and show abnormal accumulation of callose.¹³ These results suggest that DEK1 may affect the properties of cell-cell contact zones in the epidermis from early in development. Considering these results, we propose a potential mechanism via which DEK1, by potentiating cell-cell communication within the epidermal cell layer, helps to maintain the expression of HD-ZIP IV TF during organ growth.

We propose that in WT plants, where cell-cell contacts are tightly regulated, ACR4- and likely other RLK-mediated signaling pathways actively regulate HD-ZIP IV-encoding gene expression and thus epidermis differentiation is maintained over time. When DEK1 activity is reduced, cell-cell contacts are impaired. We propose that this impedes the accumulation, movement and/or perception of ligands involved in RLK-mediated intercellular signaling pathways, leading to a loss in the maintenance of HD-ZIP IV expression and cell differentiation (Figure 1). Our model show striking similarities to the situation in animals where the regulation of cell-cell adhesions it has been shown to have a strong impact on embryonic development and maintenance of tissue differentiation.²¹⁻²³

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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