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Article Addendum

Arabidopsis seed germination responses to osmotic stress involve the chromatin modifier PICKLE

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***pkl* mutant seed germination is hypersensitive to ABA treatment due to abnormally high and persistent *ABI3* and *ABI5* expression. PKL, a putative chromatin modifier, is instrumental to associate *ABI3* and *ABI5* with silent chromatin in response to ABA. Thus, PKL prevents exaggerated germination arrest responses by shutting off *ABI3* and *ABI5* expression in response to mild stresses.**

A key feature of land plant's success during evolution lies on an astonishingly bold adaptation: the ability to produce seeds, a structure encapsulating plant embryos while keeping them in a metabolically inert and osmotolerant state. The phytohormone abscisic acid (ABA) plays a key role to establish osmotolerance in the seed by promoting the expression of transcription factors such as *ABI3* and *ABI5*, which in turn promote the expression of genes encoding osmotolerance proteins.[1,2] As a result, seeds are able to withstand the harshest stresses (e.g., cold, hot or dry environments) until the proper environmental conditions become available to pursue development. The first step towards the adult phase of the plant is that of seed germination, a process whereby the embryo utilizes available food stores to transform itself into a photosynthetically active, and hence autotrophic, young seedling.

Seed germination cannot take place in absence of water. Given that the embryo abandons a highly protected state, it is not surprising that plants have evolved the capacity to arrest germination. During the time interval between the seed's first exposure to water and cotyledon greening (about two days), a sudden osmotic stress arrests germination and promotes osmotolerance.[1]

Environmental conditions determine the levels of two phytohormones gibberrelic acid (GA) and abscisic acid (ABA) that exert antagonistic influences on seed germination.[3,4] GA, synthesized shortly upon seed imbibition, is strictly necessary to initiate germination whereas ABA triggers the germination arrest and osmotolerance responses. GA and ABA levels are inversely coupled: high ABA levels are associated with lower GA levels whereas high GA levels are associated with lower ABA levels (reviewed in refs. 3 and 4). Thus, the possible developmental outcomes upon seed imbibition lie between two extreme states: germination is prevented (low GA, high ABA) or unhampered (high GA, low ABA). Both hormones exert their influence by modulating the expression of repressors of seed germination. GA binds to a specific receptor, which stimulates the destruction of RGL2, the main DELLA factor repressing seed germination.[5] ABA stimulates the accumulation of ABI3 and ABI5, which are necessary to block germination in addition to their role in conferring osmotolerance.[6]

Thus, in a nutshell, seed germination can be seen as a developmental transition whose outcome depends on the GA and ABA levels imposed by the environment. In turn, GA- and ABA-dependent signaling pathways determine the amounts of the repressors of seed germination.

In *Arabidopsis thaliana*, mutations in *PICKLE* (*PKL*), encoding a putative chromatin modifier, result in abnormally persistent expression of embryogenesis genes in the adult plant.[7,8] In particular, expression of *LEC1* and *FUS3*, two early embryogenesis genes whose expression is permanently shut off during mid-embryogenesis, is transiently reactivated during *pkl* mutant seed germination. *LEC1* and *FUS3* encode transcription factors that orchestrate major embryonic programs.[9] The trademark phenotype of *pkl* mutant plants, which also gives them their name, is their swollen primary roots containing embryonic lipids, a likely consequence of the expression of embryonic transcription factors such as *LEC1* and *FUS3*.[7,9] A remarkable observation is that under normal conditions, the penetrance of this phenotype is rather low (1 to 10%). However, it can be rendered fully penetrant by inhibiting GA synthesis during the first two days after seed imbibition but not thereafter. In contrast, excess GA in the germination medium completely eliminates "PICKLE" roots.[7] Thus, it appears that GA signaling represses embryonic differentiation, in part via chromatin remodeling changes mediated by PKL, in addition to its well known role in promoting seed germination.

Consistent with this hypothesis, it is known that embryogenesis is associated with repression of GA-signaling, a process promoted by ABA.[10] Thus, given the antagonism between GA and ABA signaling also occurring during germination, the fact that GA levels determine the frequency of *pkl* plants expressing embryonic traits could also reflect alterations in ABA-dependent responses.

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In a recent report, we investigated this possibility by asking whether PKL is necessary (1) to mediate seed germination responses to ABA and (2) regulate *ABI3* and *ABI5* expression.[10]

Under normal germination conditions (i.e., absence of ABA treatment), *ABI3* and *ABI5* mRNA and protein levels normally decrease from peak levels in dry seeds. However, *pkl* mutant seeds had a slightly delayed disappearance of *ABI3* and *ABI5* expression but this was not sufficient to significantly delay *pkl* germination relative to WT.[11] The state of the chromatin associated with *ABI3* and *ABI5* was assessed by quantitative ChIPs to measure the methylation levels in Lys9 and Lys27 at histone H3 (H3-K9 and H3-K27). High methylation levels of these histones are associated with silent chromatin. Small, but statistically significant, differences were apparent between WT and *pkl* plants that were consistent with *ABI3* and *ABI5* promoter DNA being less associated with silent chromatin.[11]

In contrast, *pkl* mutant seeds imbibed in presence of low ABA concentrations displayed hypersensitive responses at the level of *ABI3* and *ABI5* expression, which, unlike in WT seeds, was abnormally persistent and elevated for several days. As a result, *pkl* mutant seed germination was markedly delayed relative to WT seeds.[11] Abnormal expression coincided with marked changes in H3-K9 and H3-K27 methylation levels indicating that the chromatin associated with *ABI3* and *ABI5* promoter DNA in *pkl* seeds was less associated with silent chromatin relative to WT seeds.[11] This was consistent with the elevated expression levels found in *pkl* mutant seeds.

Thus, ABA-dependent signals seem to recruit PKL to influence the state of the chromatin associated with *ABI3* and *ABI5*. PKL would act to moderate the plant's response to mild osmotic stresses by limiting *ABI3* and *ABI5* expression levels, thereby preventing a maladapted growth arrest. PKL appears to play an integrative role in a complex situation of hormonal competition, regulating both GA- and ABA-dependent responses: it fine-tunes the pace of seed germination in response to ABA and maintains embryonic characters silent in response to GA.

PKL is homologous to CHD3 proteins (Chromodomain Helicase DNA-binding). In animals, the importance of CHD proteins during development has been established in several studies in mammals and Drosophila.[12] CHD proteins are components of Mi-2/NuRD chromatin remodeling complexes that regulate the changes in gene expression accompanying developmental transitions. They remodel the chromatin associated with genes into a state that is non permissive with gene transcription.[13] For example, in *C. elegans* a NuRD complex is critical to repress germline gene expression during differentiation of embryonic somatic cells.[14]

PKL's discovery suggested that chromatin remodeling by Mi-2/NuRD complexes during development transitions is conserved between animals and plants. However, this proposition needs to be put in the perspective of the different developmental strategies present in animals and plants. In animals, organogenesis tends to occur during discrete periods of the life cycle and is mostly determinate, i.e., invariant in the face of environmental changes. However, animal developmental programs are coordinated by internal parameters such as hormone levels. In contrast, plant development tends to be indeterminate, i.e., it responds to the environment met by the plant. Developmental plasticity allows plants, which are sessile organisms, to optimize survival in response to changes in the environment. Ultimately, plants interpret these changes through alterations in internal parameters, including changes in hormone levels. Thus, understanding how PKL functions in plants is of particular biological interest because: firstly, it allows comparing chromatin changes mediated by Mi-2/NuRD complexes between plants and animals and secondly, it provides an opportunity to understand how Mi-2/NuRD complexes may be recruited in contexts where the developmental outcome is indeterminate. Investigating how ABA and GA levels modulate *PICKLE* expression and product activity should identify novel protein partners and mediators of hormonal changes. This could help better understand how Mi-2/NuRD complexes exert their function in animals.

References

1. Lopez-Molina L, Mongrand S, Chua NH. A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. Proc Natl Acad Sci USA 2001; 98: 4782-7.
2. Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua NH. ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. Plant J 2002; 32:317-28.
3. Olszewski N, Sun TP, Gubler F. Gibberellin signaling: biosynthesis, catabolism, and response pathways. Plant Cell 2002; 14:61-80.
4. Nambara E, Marion Poll A. Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol 2005; 56:165-85.
5. Sun TP, Gubler F. Molecular mechanism of gibberellin signaling in plants. Annu Rev Plant Biol 2004; 55:197-223.
6. Fedoroff NV. Cross-talk in abscisic acid signaling. Sci STKE 2002; 10.
7. Ogas J, Cheng JC, Sung ZR, Somerville C. Cellular differentiation regulated by gibberellin in the *Arabidopsis thaliana* pickle mutant. Science 1997; 277:91-4.
8. Ogas J, Kaufmann S, Henderson J, Somerville C. PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in Arabidopsis. Proc Natl Acad Sci USA 1999; 96:13839-44.
9. Dean Rider S, Jr., Henderson JT, Jerome RE, Edenberg HJ, Romero Severson J, Ogas J. Coordinate repression of regulators of embryonic identity by PICKLE during germination in Arabidopsis. Plant J 2003; 35:33-43.
10. Curaba J, Moritz T, Blervaque R, Parcy F, Raz V, Herzog M, Vachon G. AtGA3ox2, a key gene responsible for bioactive gibberellin biosynthesis, is regulated during embryogenesis by LEAFY COTYLEDON2 and FUSCA3 in Arabidopsis. Plant Physiol 2004; 136:3660-9.
11. Perruc E, Kinoshita N, Lopez-Molina L. The role of chromatin-remodeling factor PKL in balancing osmotic stress responses during Arabidopsis seed germination. Plant J 2007; 52:927-36.
12. Marfella CG, Imbalzano AN. The Chd family of chromatin remodelers. Mutat Res 2007; 618:30-40.
13. Ahringer J. NuRD and SIN3 histone deacetylase complexes in development. Trends Genet 2000; 16:351-6.
14. Shin TH, Mello CC. Chromatin regulation during *C. elegans* germline development. Curr Opin Genet Dev 2003; 13:455-62.