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Article Addendum

Identification of a putative receptor-ligand pair controlling cell separation in plants

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Cell separation events are important throughout the lifespan of a plant. To assure that the plant's integrity is not compromised, such events, which depend on cell wall degradation, have to be tightly controlled both in time and space. The final step of floral organ abscission in Arabidopsis is controlled by *INFLORESCENCE DEFICIENT IN ABSCISSION* (*IDA*), in that mutation of *IDA* causes a block in abscission. Overexpression results in early abscission of floral organs. In a recent article we show that this is also the case when overexpressing the related IDA-LIKE (IDL) proteins, indicating a degree of functional redundancy. Based on gene swap and deletion constructs introduced in the *ida* mutant and synthetic peptide assays we demonstrated that the conserved C-terminal motif (EPIP) of IDA and IDL1 was sufficient to replace IDA function. This function is dependent on the presence of the receptor-like kinases (RLK) HAESA (HAE) and HAESA-LIKE2 (HSL2), suggesting that an IDA peptide acts as a ligand interacting with these receptors. Our study further revealed that the five *IDL* genes are expressed at various sites where cell separation takes place. We suggest that the IDL proteins constitute a family of ligands that act through RLKs similar to HAESA and control cell separation at different sites and development stages during the life of the plant.

Introduction

Cell-to-cell interactions are essential for the development of multicellular organization and for the function of most organ systems. All multicellular organisms have evolved mechanisms to perceive and respond to extracellular chemical signals, including endogenous hormones, small peptides and external cues from the environment.[1] In plants adjacent cells are joined together by a cellulose wall and signaling between cells pass through plasmodesmata[2,3] or via receptor-ligand interaction on the cell surface.[4] The cell wall provides plants with strength and rigidity but also constrains the activity and autonomy of individual cells.[5-8] Most cells remain attached to their neighbors throughout their life, but during a number of processes in the life cycle of a plant (e.g., germination, formation of stomata, organ and seed shedding) it is crucial that separation between neighboring cells takes place.[9]

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IDA and the IDL Proteins are Signaling Molecules

IDA has been shown to control floral organ cell separation (abscission),[10-12] given that specialized abscission zone (AZ) cells are present.[13] IDA and the five Arabidopsis IDL proteins have an N-terminal signal peptide (SP) that has been shown to direct GFP-fusion proteins of IDA, IDL1 and IDL3 to the periphery of onion cells.[10,14] Their small size and extra-cellular localization made us suggest that these proteins are ligands involved in cell-to-cell communication.[10] The SPs of IDA and the IDLs should direct the proteins to the apoplastic space, by the default pathway for soluble plant proteins,[15] and enable a potential receptor interaction. In agreement with this, we show that plants harboring constructs designed to overexpress IDA or IDL1 without the SP displayed none of the phenotypes observed in *35S:IDA* and *35S:IDL1* plants.

Receptor-ligand Interaction

We have previously proposed that IDA could be the ligand of the leucine-rich repeat (LRR)-RLK HAESA (HAE).[10,16] In our recent paper, we report that the double mutant of *HAE* and *HAESA-LIKE2* (*HSL2*) has a block in floral abscission, a phenotype similar to that of *ida*. We also demonstrate that *hae hsl2* is epistatic to *35S:IDA*, providing genetic evidence that *IDA*, *HAE* and *HSL2* act in the same pathway. We hypothesize that IDA can signal through both HAE and HSL2, but we can not say which of the two, or if both, receptors normally relay the IDA signal.

When ectopically expressed all the IDL proteins were capable of inducing floral organ abscission. These results indicate that the IDL proteins are able to trigger abscission through the same cellular mechanism as IDA, and that the IDL proteins may function through similar signaling pathways. However, promoter-reporter gene constructs indicate that the *IDL* genes are expressed in diverse tissues and not only in the floral organ AZs, suggesting that their normal functions differ from that of IDA. Therefore it is probable that the putative IDL ligands can exert their effects both through an

IDA receptor and their native receptors and perhaps other non-native receptors. Functional redundancy is common in higher plants, and it has been shown that several members of the CLE family can rescue the *clv3* loss-of-function phenotype.[17]

Functional Redundancy

To study the degree of functional redundancy under endogenous biological conditions, we investigated whether the IDL proteins could rescue the *ida* mutant phenotype when expressed under the control of the *IDA* promoter. Only IDL1, which has the highest overall sequence similarity to IDA, was capable of replacing IDA. The other IDLs showed a limited ability to substitute for IDA. This might be explained by a threshold model based on the assumption that IDA is interacting with a receptor at the cell surface. In the presence of a suboptimal IDL peptide, the number of signaling complexes might be reduced to a lower-than-the-threshold number, due to reduced ligand-receptor binding affinity compared to the native IDA-receptor interaction. When the concentration of the proteins is high enough, as it is when overexpressed, the reduced affinity for the receptor could be circumvented by an increase in ligand concentration.

The Functional Domain is Found in EPIP

The only conserved region between IDA and the IDL proteins is a C-terminal motif called EPIP. Thus, the functional domain of IDA is thought to be contained within the EPIP domain. The replacement of the EPIP domains of non-functional IDL with that of IDA turned these proteins into functional proteins, substantiating this idea. In addition, a deletion analysis demonstrated that all constructs containing the IDA EPIP motif rescued the *ida* mutant, whereas the construct lacking the EPIP domain did not. Furthermore, synthetic IDA and IDL1 EPIP peptides were able to rescue the *ida* mutant. However, these peptides could not induce abscission in *hae hsl2* mutant flowers, suggesting that the EPIPs interact with these receptors.

It is tempting to speculate that the EPIP domains, like the CLE domain of CLV3,[17] are released as functional peptide ligands from protein precursors. Although no obvious cleavage recognition site has been found in IDA or the IDL proteins, mobility shifts, consistent with a distinct C-terminal processing, was detected using extracts from cauliflower meristem. Future studies will hopefully reveal whether this processing reflects the situation in Arabidopsis and delineate the shortest IDA and IDL peptides necessary for biological function. Comparing the EPIPs of IDA and IDL1 to the less functional IDL EPIPs reveals four residues that are only common to IDA and IDL1. Our hypothesis is that one or several of these amino acid residues might be crucial for IDA-EPIP function.

Conclusions

Assuming that IDL peptides act as ligands, differences found in their EPIP domains could reflect a preference of individual IDL members for interaction with different but similar receptors. Based on the phenotypic similarities resulting from overexpression of the *IDA* and *IDL* genes, LRR-RLKs closely related to HAE and HSL2 may be postulated as signaling partners of the IDL proteins. The exciting fact that *IDL* genes were found to be expressed at sites where cell separation occurs,[9] may indicate that these proteins are signals inducing cell separation and/or cell wall modification during different developmental stages throughout the life span of a plant.

Note added in proof

That *35S:IDA* is epistatic to *hae hls2* has recently also been shown by Cho et al. (Proc Natl Acad Sci USA 2008; Sept 22).

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