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The Regulation of Root Hair-specific Expansin Genes

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The root hair provides a major entering spot for the symbiotic legume rhizobia. It is obvious that dynamic cell wall modification occurs in the plant root hair during the early microbe invasion. Expansins are non-destructive cell wall-modifying proteins that are involved in cell growth and differentiation. Among about 40 expansin genes in Arabidopsis, two expansin genes are expressed specifically in the root hair cell. Orthologous genes of this Arabidopsis root hair expansins have been found in other Brassica members, rice, and *Medicago truncatula* (a legume). In this review, I discuss the probable function of expansins during the early symbiotic process between the root hair and microbes and the regulation of root hair expansin genes in a comparative approach.

Keywords **:** ethylene, expansin, nodulation, promoter analysis, root hair

Introduction: The root hair and symbiotic processes

Root hairs are the important infectious entry by soil microbes such as rhizobia, actinomycete, and mycorrhizal fungi. The early interactions between the root hair and nitrogen-fixing rhizobia have been well characterized. The earliest signaling molecules secreted by rhizobia are lipochito-oligosaccharides, the Nod factors. Rhizobia and Nod-factors alter the polarity and thus the growing pattern of the root hair, resulting in a deformation of the root hair such as distortions, branching, and curling. This deformed part of the root hair is the place where rhizobia can infiltrate into the cell wall and the plant cytoplasm.

The entry of rhizobia occurs through the hair cell wall part that consists of non-crystalline cellulose (Mateos et al., 2001). This process necessarily accompanies the cell wall modification by plant enzymes induced by rhizobial Nod factors and the complete wall degradation by rhizobial wall-degrading enzymes (Munoz et al, 1998). Among these wall-degrading enzymes are cellulases and pectinases, which are found in *Frankia* (an actinomycete) as well as in Rhizobia (Seguin et al., 1989).

Cell wall modification can be accomplished by the functions of diverse wall proteins including not only degrading enzymes as aforementioned but also non-destructive wall-modifying proteins. Expansins are non-destructive wall modifying proteins that are capable of loosening and modifying the cell wall by disrupting hydrogen bonds between cellulose microfibrils and matrix polymers (Cosgrove, 1999).

Expansins occur as a multigene family in land plants. The model plant Arabidopsis harbors approximately 40 expansin genes of which two expansins show the root hair cell-specific expression pattern (Cho and Cosgrove, 2002). Here, I suggest the probable function of expansins during the symbiotic process of soil microbes and in root hair formation and describe the regulation of two root hair-specific expansin genes.

Expansins: The non-destructive cell wall modifying proteins

Expansins were discovered in efforts to find the wall loosening factor which could extend isolated plant cell walls in response to acids (pH<6). Acids can induce elongation of excised plant stems such as hypocotyls and coleoptiles, and this has been called acid growth. This phenomenon could be reproduced with isolated, frozen, pressed cell wall specimens from the stems. Attention was then focused to the cell wall factor that is capable of loosening the cell wall in acidic conditions. This factor must have been proteinous because the acid-induced cell wall extension was abolished by heating or protease treatments (Cosgrove, 1989). Using the creep assay system,

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where fractionated cell wall proteins were tested if they could induce extension of the heat-inactivated cell wall, in 1992, the first expansin proteins were isolated from cucumber hypocotyls (McQueen-Mason et al., 1992). Together with the following characterization of expansin proteins from rice stems (Cho and Kende, 1997a), it was shown that the function of expansins are highly conserved in deeply diverged mono- and dicotyledonous cell walls.

Expansins do not show degrading activity with most cell wall components. Rather, expansins are likely to loosen or modify the cell wall by disrupting hydrogen bonds bridging between cellulose microfibrils and hemicellulosic matrix polymers (McQueen-Mason and Cosgrove, 1994). This function of expansins is very unique because any other wall-hydrolytic enzymes such as cellulases and pectinases could not induce extension of isolated cell walls and because expansins are the only wall-loosening factors ever known in whole wall-surrounded organisms. However, structurally, expansins share some similarity with certain fungal or bacterial cellulases, raising a curious question how the biochemistry between expansins and cellulases has diverged into different destinations.

Further studies demonstrated that all major branches of the plant kingdom, from bryophytes to seed plants, carry conserved expansin gene family members (Li et al., 2002). Among unicellular eukaryotes, *Dictyostelium* and *Chlamydomonas* have genes encoding the strictly conserved protein motifs in expansins, and there are some bacterial proteins including these motifs. However, the function of these expansin motif-containing proteins from lower eukaryotes and prokaryotes remains to be elucidated.

The expression patterns of expansins are highly correlated with plant cell growth and possibly tissue differentiations that accompany vigorous wall modifications (Cho and Kende, 1997b; Cho and Kende, 1998). Moreover, suppression or overexpression of expansins is able to alter plant organ growth and organ abscission (Cho and Cosgrove, 2000). The latter case could be of interest in the context of microbe symbiosis. Cell wall modifications and degradations are prominent phenomena during the abscission process (Gonzalez-Carranza ZH et al., 1998). This indicates that the non-destructive biochemistry of expansins contributes for the destructive physiological process, possibly by helping or enhancing the activities of wall degradation enzymes.

In relevance with the symbiotic interaction between plant roots and soil micro organisms, it is an interesting question whether the symbiotic microbes possess expansin-like proteins and whether their functions are involved in the symbiotic processes. As briefly mentioned in Introduction, the early symbiotic process between two kingdoms includes intensive modifications of the plant cell wall. It was already suggested that pectinase and cellulase activities could be needed to form the hole for microbe entry in the plant root hair cell. Expansin activities might be involved in this process with other wall degradation enzymes as they do during organ abscission. These expansin activities may come from either plant root hairs or the microbes, or from both.

Root hair formation and patterning

The root hair is the protrusion of root epidermal cell and occurs in all vascular plants. In Brassicaceae including Arabidopsis, the root hair occurs only in the epidermal cell that overlies on two underlying cortical cells (type 3), which gives rise to a longitudinally striped distribution of root hair and non-root hair cells. The fate to hair or non-hair cells is determined by interactions of series of transcription factors (for review, see Schiefelbein, 2003). Briefly, WEREWOLF (WER) induces expression of CAPRICE (CPC) and GLABRA2 (GL2) in the non-hair cell position. GL2 is the inhibitor of root hair morphogenesis. CPC moves to the hair cell position and inhibits the expression of WER and GL2, which gives rise to root hair formation in the hair cell. However, it remains to be elucidated what kind of positional cue determines the cell fate and which genes are inhibited by GL2.

Besides this striped type of root hair distribution, there are two more types of root hair patterning (Clows, 2000). In Grass family, the root hair rises from the smaller cell after asymmetric vertical division of the epidermal cell (type 2), suggesting that unequal allocation of cytoplasm is involved in this cell fate determination. Many other dicotyledons (including legumes) and monocotyledons show the random distribution type of root hairs (type 1). Type 1 could be derived from the loss of some steps needed for type 2 or type 3, or it also could be established by another fate-determining mechanism.

Three different types of root hair patterning lead us to think that fate-determining machinery, such as the WER/CPC/GL2 pathway in Arabidopsis, governs the process for root hair formation. This is true, but the fate-determining machinery is not all that determines root hair formation. Hormones and environmental factors such as ethylene, auxin, water stress, and nutrient necessities can affect root hair formation via a distinctive pathway from that of fate-determining machinery (Cho and Cosgrove, 2002). The hormonal and environmental effects on root hair formation are held not only in the Brassica type root hair pattern but also in other two types of hair patterning. This implies that the regulation of root hair morphogenesis itself is an independent intracellular process, whereas root hair patterning on the root epidermis is a heritage of intercellular interactions. Therefore, it can be hypothesized that the

regulation of root hair-specific genes for cellular morphogenesis is conserved among plant groups despite their different root hair patterning tools. This idea can be useful to study root hairs of important crop plants, such as symbiotic legumes, by adopting the knowledge from well-characterized model plants.

Root hair-specific expansin genes and their regulation

The genome of Arabidopsis counts about 40 expansin genes which show cell type-specific expression patterns. Two of these, *AtEXP7* and *AtEXP18*, are expressed only in the root hair cell (Cho and Cosgrove, 2002). These genes start to express immediately before the root hair initiation. The root hair bulges out only from the apical part of an epidermal cell, indicative of a polarized cellular process. Thus this process should accompany localized cell wall loosening at the hair-bulging point and also polarized cellular secretions. Root hair expansins can be required for this initial process of root hair emergence.

The Arabidopsis root hair expansin genes are consistently regulated by the upstream transcription factors representative of the fate determiners. However, these genes also can be regulated by ethylene, auxin and water stress, which is independent of the fate-determining pathway (Cho and Cosgrove, 2002).

It was an intriguing question what the *cis*-elements for the root hair specificity and the responsiveness for hormones and environmental factors are and whether these factors interact with independent *cis*-elements or they converge into a single *cis*-element. Deletion, substitution, and gain-of-function promoter analyses reveal that the root hair specificity and the responsiveness to hormone/environment factors are likely to be determined by a single transcription binding site. This suggests that the pathways from both fate determination (root hair specificity) and hormone/environment signals share a common transcription factor to switch on the root hair-specific expansin genes.

Yeast one-hybrid screening of the Arabidopsis root cDNA library using the *cis*-element as bait revealed that an AP2 domain-containing putative transcription factor can recognize this promoter element (H-T Cho, unpublished result). This transcription factor probably can merge the pathways from both fate determiners and hormone/environment factors. Further study to show this possibility is under process in my group.

Considering that the root hair morphogenetic process and the fate determination can be separated, we hypothesize that the regulation of root hair-specific expansin genes can be achieved by the common manipulator even between different hair-patterning taxa. To satisfy this hypothesis, it is necessary to find whether other hair-patterning groups have the orthologs of Arabidopsis root hair expansins and whether the promoters from these orthologs contain the same *cis*-element for hair specificity. *AtEXP7* orthologs have been found from cabbage, Chinese cabbage, radish, rice, and *Medicago truncatula* (a legume). Rice belongs to type 2 root hair patterning and *M. truncatula* to type 1. The 9- nucleotide core *cis*-element for the root hair specificity has been found in cabbage, Chinese cabbage, and rice orthologs. It is highly intriguing whether the promoters or *cis*-elements from these orthologs are able to direct the root hair specificity in its own root and in the root from other patterning species. The study on this topic is proceeding in my group, and the results would shed light on comparative approaches to and evolutionary understanding of cell differentiation processes.

Perspectives

Root hairs contribute for most of the root surface area and thus are very important for symbiotic interactions with soil micro organisms as well as for absorption of water and nutrients. Manipulating gene expression in a root hair-specific manner could be greatly useful for both research and practical purposes. The universal regulatory mechanism for root hair gene expression can also be helpful for application of the knowledge from one species case to the other species cases. Expansins are unique wall-modifying proteins that might be involved in symbiotic processes between plant root hairs and soil microbes. However, it remains to be understood if the symbiotic microbes have the equivalents of expansins and whether and how plant root hair expansins function during the symbiotic interactions.

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