

## **START domains in lipid/sterol transfer and signaling in plants**

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### **Abstract**

Steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domains were first identified in cholesterol-binding StAR orthologs from mammals. They are also found in plant proteins, where they are predicted to mediate transport and signaling of lipids. START domains in combination with the homeodomain (HD) DNA-binding motif are unique to plants. Recent genetic analysis of the HD-START transcription factor family from *Arabidopsis* has revealed roles in development. Strikingly, the corresponding mutant phenotypes are similar to those found in a small set of sterol biosynthesis genes. Thus, START domains in HD transcription factors may control gene expression by sterol binding, linking sterol metabolism to cell differentiation in plants. Another family of START domain proteins contains a pleckstrin homology (PH) domain that is implicated in phospholipid signaling. One member of this family has recently been shown to function in disease resistance, and a model for the role of its START domain in intermembrane transfer of ceramide in programmed cell death (PCD) is proposed.

### **Introduction**

START domains are ~200 amino acid lipid/sterol binding modules that are conserved from animals to plants (Ponting and Aravind 1999; Schrick *et al.* 2004a). The prototype START domain is found in mammalian StAR, a transporter of cholesterol. In humans, mutation or misexpression of START proteins is linked to inherited disorders, autoimmune disease and cancer (reviewed in Alpy and Tomasetto 2005). Three mammalian START domain structures have been solved and shown to form a hydrophobic tunnel that binds a single lipid/sterol ligand. In addition to sterols, ligands for human START domains include phospholipids and sphingolipids. Thus far, ligands for plant START domains have not been identified.

Comparison of major sequenced genomes revealed that START domains are amplified in plants (Schrick *et al.* 2004a). The human and *Arabidopsis* genomes contain 15 and 35 START domain genes, respectively. The majority of *Arabidopsis* START domain proteins are members of a plant-specific HD transcription factor family that is conserved in rice. *Arabidopsis* contains 21 HD-START proteins, 16 of which are characterized by a HD amino-terminal to a plant-specific leucine zipper with an internal loop, referred to as class IV HD Leu-zipper (HD-ZIP). In the smaller subfamily of five proteins known as class III HD-ZIP, the HD precedes a classic basic-region leucine zipper, and members of this family also contain a carboxy-terminal PAS-like MEKHLA domain (Mukherjee *et al.* 2006). Another *Arabidopsis* family of four START domain proteins contains a PH domain together with a plant-specific domain of unknown function (DUF1336), and one protein contains the START-DUF1336 combination alone. The remaining nine START domain proteins in *Arabidopsis* are referred to as START-domain minimal proteins as these appear to be comprised of START domain only. *Arabidopsis* START domain proteins that have been characterized by genetic analysis will be discussed below. Phenotypes have been uncovered in classical genetic screens, as

well as by systematic analysis of T-DNA insertion mutants. In light of recent findings, possible scenarios for START-mediated lipid signaling are proposed.

### **Plant sterols as candidate ligands for START domains in HD transcription factors**

Several HD-START genes from the class IV HD-ZIP family play roles in layer-specific cell differentiation (reviewed in Schrick *et al.* 2004a). *Arabidopsis thaliana* *MERISTEM LAYER1* (*ATML1*) and *PROTODERMAL FACTOR2* (*PDF2*) exhibit redundant functions in epidermal differentiation. *GLABRA2* (*GL2*) is required for trichome and non-root hair cell fate in the epidermis. Mutations in *ANTHOCYANINLESS2* (*ANL2*) affect the cellular organization of the root and anthocyanin accumulation, indicative of a role in sub-epidermal cell identity. In addition, T-DNA insertions in the other family members, *HOMEODOMAIN GLABROUS1-12* (*HDG1-12*), have been characterized recently (Nakamura *et al.* 2006). Mutations in *HDG11* result in excess branching of trichomes, which is enhanced in combination with *hdg12*. No obvious phenotypes were observed for other *HDG* genes, except that *hdg3 atml1* and *hdg3 pdf2* double mutants exhibit defects in cotyledon patterning. The five genes from the class III HD-ZIP family, *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), *REVOLUTA/INTERFASCICULAR FIBERLESS1* (*REV/IFL1*), *CORONA* (*CNA*) and *ATHB-8*, have multiple and partially overlapping roles in development, including embryonic patterning of the shoot meristem and vasculature, and organ polarity (Prigge *et al.* 2005).

Genetic evidence for the role of sterols in plant development stems from the identification of three *Arabidopsis* sterol biosynthesis mutants: *fackel* (*fk*), *cephalopod/sterol methyltransferase 1* (*cph/smt1*) and *hydra1* (*hyd1*), which exhibit cell division and expansion defects along with patterning defects in embryogenesis (Diener *et al.* 2000; Jang *et al.* 2000; Schrick *et al.* 2000; 2002; Souter *et al.* 2002). That all three mutants represent lesions upstream of brassinosteroids (BR), coupled with the inability of BR to rescue their defects, points towards the direct involvement of sterols in signaling pathways for growth and development. A current strategy to elucidate the underlying sterol signal transduction pathways is to identify sterol-binding proteins in plants.

HD-START transcription factors are excellent candidates to be controlled by binding to sterols. Consistent with this idea, mutations in sterol biosynthesis and HD-START genes share common phenotypes. This is exemplified by vasculature defects in sterol biosynthesis mutants that are similar to defects observed in *rev/ifl1*, *phb* and *phv* mutants. Moreover, sterol biosynthesis mutants exhibit ectopic shoot meristems (Schrick *et al.* 2000; Schrick *et al.* 2002), an unusual feature that is also reported for *phb* mutants (McConnell and Barton 1998). Further, trichome spacing and branching, which are affected in *fk* and *hyd1* (Schrick *et al.* 2004b), is also seen in plants overexpressing *GL2* (Ohashi *et al.* 2002), and in *hdg11* mutants (Nakamura *et al.* 2006), respectively.

Since HD-START proteins are transcription factors, a major prediction is that START, by binding to sterols, regulates the gene expression analogously to steroid hormone receptors from animals (Ponting and Aravind 1999; Schrick *et al.* 2004a). This mechanism would allow lipid metabolism to be linked to cell differentiation in plants. Changes in the activity of a HD-START transcription factor could be controlled by a ligand-binding induced conformational change that affects DNA binding affinity or interaction with accessory proteins. Alternatively or in addition, ligand binding may affect transport or sequestration of the transcription factor to the nucleus.

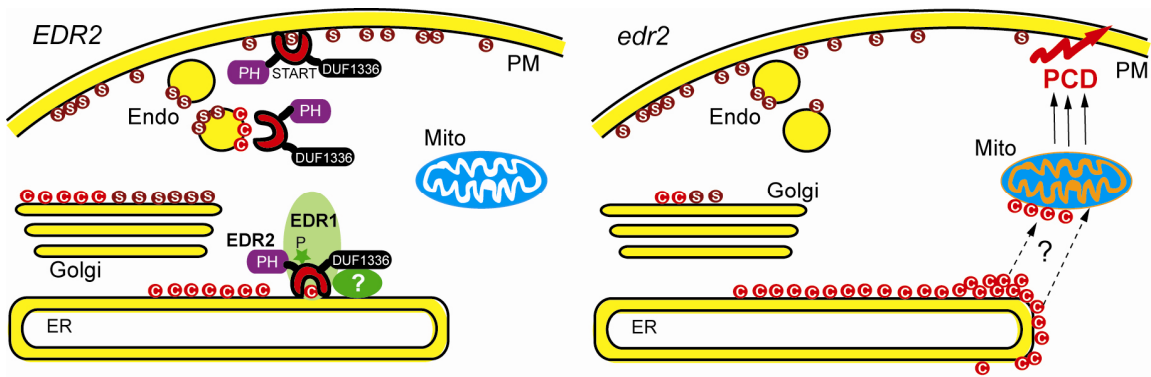
### **Lipid signaling in plant defense responses: The EDR2 - CERT paradigm**

Genetic analysis of enhanced disease resistance (EDR) to the powdery mildew *Erysiphe cichoracearum* in *Arabidopsis* led to the identification of mutations in *EDR2*, another type of START domain gene (Tang *et al.* 2005). The predicted protein also contains a PH domain and a highly-conserved plant-specific domain, DUF1336. Loss-of-function mutations in *EDR2* confer enhanced resistance to powdery mildew, pointing towards *EDR2* as a negative regulator of pathogen-induced resistance. Upon infection, *edr2* mutants exhibit salicylic acid-dependent hypersensitive necrosis response (HR) coinciding with the arrest of fungal growth, increased callose deposition, and enhanced pathogen-related (PR-1) gene expression. The *edr2* phenotype strongly resembles that of *edr1*, and the double mutant is indistinguishable from either single mutant with respect to both HR and ethylene-induced senescence (Tang *et al.* 2005), indicating their function in a common pathway. *EDR1* encodes a CTR1-like protein kinase (Frye *et al.* 2001) having Ser/Thr kinase activity (Tang and Innes 2002). One prediction is that *EDR2* is a phosphorylated target of *EDR1* kinase. Phosphorylation has been shown to be important for START domain function in at least one case from animals (Arakane *et al.* 1997). Alternatively, *EDR1* may phosphorylate a cofactor that is required for *EDR2* function.

The human ceramide transport protein CERT contains a PH and a START domain (Hanada *et al.* 2003) and hence exhibits modular similarity to *EDR2*. CERT facilitates the intermembrane non-vesicular trafficking of ceramide from the endoplasmic reticulum (ER) to the Golgi apparatus, where it is converted to sphingomyelin. Ceramide is the backbone molecule of the sphingolipids, which are abundant components of the plasma membrane in eukaryotes. The START domain of CERT specifically binds ceramides while the PH domain mediates targeting to the Golgi compartment by binding phosphatidylinositol 4-monophosphate (PtdIns4P). In addition, CERT also contains a middle region that associates with the ER (Loewen *et al.* 2003). The transport assay for CERT function requires ATP (Hanada *et al.* 2003), consistent with the idea that ceramide binding and release is regulated by phosphorylation. Corroborative evidence for CERT analogy to *EDR2* arises from a recent discovery that the PH domain of *EDR2* binds to PtdIns4P, together with the observation that an *EDR2*:GFP protein localizes to the ER, plasma membrane and endosomes (S. Somerville, personal communication). Thus, *EDR2*, like CERT, appears to mediate intermembrane trafficking.

A model for the role of the *EDR2* START domain in disease resistance is presented in Figure 1. It is hypothesized that in *edr2* mutants, a defect in START-mediated lipid trafficking results in the accumulation of ceramide which triggers the HR, a form of programmed cell death (PCD), upon pathogen attack. Genetic evidence linking aberrant levels of ceramides to PCD in plants comes from the characterization of *Arabidopsis* mutants for ceramide kinase, *accelerated cell death 5 (acd5)* (Liang *et al.* 2003) and sphingosine transfer protein, *acd11* (Broderson *et al.* 2002).

A role for *EDR2* in the mitochondria was previously suggested, based on a predicted amino-terminal mitochondrial targeting sequence (Schrack *et al.* 2004a; Tang *et al.* 2005). However, its subcellular localization to the ER, plasma membrane and endosomes is contrary to this idea. Nonetheless, this does not discount the involvement of mitochondria. There are numerous reports of mitochondrial defects leading to PCD in animal systems, and ceramides are implicated as effectors (reviewed in Kroemer and Reed, 2000; Green 2005). Moreover, ceramide-induced loss of mitochondrial membrane



**Figure 1.** Defective START domain trafficking of ceramide in a model for disease resistance of *edr2*. Ceramides are synthesized in the ER and EDR2 shuttles ceramides (C) and derived sphingolipids (S) between membrane compartments via its START domain. EDR1 kinase is postulated to phosphorylate EDR2, or alternatively an unknown cofactor, to control START-domain binding and release of ligand. In *edr2* mutants, ceramides accumulate in the ER, priming mitochondria membrane permeabilization (MMP) via an unknown mechanism. Infection with pathogen results in increased MMP that leads to PCD. Endo, endosomes; ER, endoplasmic reticulum; Mito, mitochondria; P, phosphorylation; PM, plasma membrane.

potential has been correlated to PCD in *Arabidopsis* protoplasts (Yao *et al.* 2004). In the proposed model for HR in *edr2* mutants, the accumulated ceramides act directly or indirectly on mitochondrial membrane permeabilization (MMP) (Figure 1). It is predicted that in *edr2* mutants, the mitochondria are primed in the initiation state and infection with the pathogen results in an increased MMP thereby triggering the onset of PCD.

EDR2 is likely to represent a redundant function, null mutations in which lower the threshold of activation for host defenses. It is striking that *edr2* mutants lack obvious developmental defects. EDR2 is a member of a small gene family of four PH-START-DUF1336 proteins in *Arabidopsis*. Its closest relative, At5g44560, displays 89% amino acid identity with EDR2. The characterization of double and/or multiple mutants is likely to reveal additional cellular functions for PH-START-DUF1336 proteins in plants.

### Perspectives

The START lipid/sterol-binding module is predicted to play a central role in lipid signaling in plants. Although START domains are amplified in plants relative to animals, a function in ligand binding has not been experimentally verified for any plant protein. Genetic analysis in *Arabidopsis* is incomplete, but so far indicates that START domain proteins of the HD-START and PH-START-DUF1336 subfamilies play vital roles in diverse processes from embryogenesis to disease resistance. Structural data for plant START domains have not been reported to date, but this would aid in identifying lipid/sterol ligands. Elucidating the roles of START domain proteins in lipid transfer and signaling represents a key step towards establishing regulatory networks that integrate stress response, metabolism and development in plants.

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