

# Unveiling the functional diversity of the alpha/beta hydrolase superfamily in the plant kingdom

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## Addresses

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## **Abstract**

The alpha/beta hydrolase (ABH) superfamily is a widespread and functionally malleable protein fold recognized for its diverse biochemical activities across all three domains of life. ABH enzymes possess unexpected catalytic activity in the green plant lineage through selective alterations in active site architecture and chemistry. Furthermore, the ABH fold serves as the core structure for hormone receptors in the gibberellin, strigolactone, and karrikin signaling pathways in plants. Despite recent discoveries, the ABH family is sparsely characterized in plants, a sessile kingdom known to evolve complex and specialized chemical adaptations as survival responses to widely varying biotic and abiotic ecologies. This review calls attention to the ABH superfamily in the plant kingdom to highlight the sparsely understood functional adaptability of the ABH fold.

## Introduction

Serine hydrolases are one of the most prevalent enzyme families constituting approximately 1% of the human genome [1]. The core catalytic machinery of these enzymes is composed of a conserved Ser/His/Asp(Asn) catalytic triad and a transition state stabilizing oxyanion hole provided by the peptide backbone [2]. The majority of these enzymes fall into the large alpha/beta hydrolase (ABH) fold superfamily, classified in 1992 [3]. This protein superfamily is found in all domains of life serving catalytic roles in primary and specialized (secondary) metabolism as esterases, thioesterases, lipases, proteases, dehalogenases, haloperoxidases, and epoxide hydrolases [4–6]. Our understanding of the catalytic and non-catalytic versatility of this family continues to expand. As enzymes, ABHs are classically responsible for the hydrolysis of ester and peptide bonds. However, ABHs also participate in the breaking of carbon-carbon bonds [7], decarboxylation reactions [8–11], and the fascinating cofactor-independent dioxygenation of heteroaromatic rings [12].

The core fold of ABHs is an 8-stranded  $\beta$ -sheet surrounded by  $\alpha$ -helices. Structural and functional variation is typically dependent on additional structural elements, often referred to as lid domains (Figure 1) [4]. The number of  $\beta$ -strands and  $\alpha$ -helical segments vary, but the intervening loops carrying the catalytic Ser, His, and Asp/Asn residues are the most conserved features defining the ABH family. Nevertheless, across all three domains of life, ABH family members often share surprisingly low sequence identity (e.g. 6.2% identity/9.3% similarity between *Pseudomonas* sp. Dienelactone hydrolase and Wheat serine

carboxypeptidase II) while maintaining a highly conserved three-dimensional core architecture (Figure 1).

Due to their sessile nature, plants rely on a diverse repertoire of specialized, often taxon-specific metabolites and sophisticated signaling systems to communicate and survive in ecosystems challenged by a myriad of biotic and abiotic factors. The adaptability of the ABH fold has allowed it to serve as an exemplary scaffold for the evolutionary “design” of additional enzyme chemistries and biological functions in the green plant lineage. Recent discoveries include hydroxynitrile lyases [7,13], polyneuridine aldehyde esterase [11], and methyl ketone synthase [8–10]. Notably, the alpha/beta hydrolase fold also functions as bona fide hormone receptors in the strigolactone, karrikin-smoke receptor, and gibberellin response pathways [14–19]. Despite mounting evidence for the importance of these enzymes in plant physiology and specialized metabolism, the *Arabidopsis thaliana* genome alone contains hundreds of uncharacterized ABH-like genes (Figure 2). Notably, only a small number of ABH structures from all green plants have been experimentally determined. This review seeks to provide a representative overview of the diverse functions of the ABH superfamily in the green plant lineage, with a focus on unique structural elements recently uncovered in plant ABHs related to unanticipated catalytic and signaling functions. Given the large number of ABHs encoded in plant genomes, future work will undoubtedly discover additional roles for ABHs in plant metabolism, growth, development, survival and overall fitness.

### **ABHs and their role in primary and specialized metabolism**

ABHs are commonly associated with housekeeping roles that participate in the breakdown and recycling of cellular metabolites, processing of external nutrients and detoxification of xenobiotics [2,20–22]. In addition, ABHs play key regulatory roles in metabolism. C-terminal peptidases, such as carboxypeptidase II, are essential for modulating protein lifetime, function, and turnover [23,24]. Notably, carboxypeptidase II from wheat served as one of the founding members of the plant ABH family [3,24]. In addition to peptidases, lipases play central roles in lipid metabolism and signaling. Phospholipases, many belonging to the plant ABH family, are key to generating chemical signals at the cell membrane [21]. Phospholipases produce second messengers, such as diacyl glycerol (DAG) and phosphatidic acid (PA) to regulate cell function and respond to environmental cues [21]. Plants also depend on lipases as catalytic hubs in the biosynthesis of the volatile and phytohormone jasmonic acid [21]. The *A. thaliana* phospholipase, *A. thaliana* DAD1-like seedling establishment-related lipase (AtDSEL), whose three dimensional structure has been elucidated, likely plays a critical role in mediating lipid mobilization during seed germination and growth [25].

### **Diversity of catalytic ABHs in the green plant lineage**

In addition to serving roles in core metabolic processes, ABHs also support a variety of unique catalytic functions for defense and hormone regulation. Salicylic acid binding protein (SABP2) is an ABH esterase that regulates responses to salicylic acid (SA), a key hormone for plant immune

responses [26–28]. SA signaling activity can be modified through carboxyl-directed methylation by a salicylic acid methyl transferase (SAMT), an S-adenosyl-*L*-methionine (SAM)-dependent methyl transferase (MT) in the SABATH family MTs [29–31]. Methylated SA (MeSA) curtails SA signaling and renders the core SA molecule more volatile and lipophilic. These properties afford the plant with a store of inactive SA to ensure rapid response to challenges by immune elicitors and possibly furnish enhanced transport of SA metabolites within and between plants. SABP2, initially described as a SA binding protein, was later shown to act as a MeSA esterase, hydrolyzing MeSA to produce active SA [32,33]. SABP2's catalytic mechanism follows the canonical ABH mechanism, outlined in Figure 3. Interestingly, the reaction product SA possesses high affinity for SABP2's binding pocket. This facile binding to the SABP active site is thought to participate in feedback inhibition to control the strength and duration of the SA response [33].

More unexpected deviations from the canonical ABH reaction scheme in green plants are typified by hydroxynitrile lyase (HNL), methyl ketone synthase 1 (MKS1), and polyneuridine aldehyde esterase (PNAE) (Figure 1). HNLs from *Manihot esculenta* and *Hevea brasiliensis*, have been studied structurally and enzymatically [7,13,34–36]. HNLs produce protective cyanide from plant phytoanticipins, cyanohydrin glucosides, during defense against herbivory [37–39]. HNLs catalyze the breaking of cyanohydrin C-C bonds to yield lethal doses of cyanide that kill chewing insects by inhibiting key enzymes of the insect electron transport chain [38]. HNL retains the typical ABH catalytic triad

(Ser80/His235/Asp207), but also depends upon a unique active site lysine residue, Lys236, that modulates the pKa of His235 and stabilizes charged reaction intermediates during substrate turnover [35,36]. The mechanism deviates significantly from standard ABHs, whereby the active site serine residue acts as a catalytic base instead of a nucleophile (Figure 3). Additionally, HNL organizes a non-canonical oxyanion hole provided by the backbone amide of Cys81 and  $\beta$ -hydroxyl group of Thr11. These evolutionary adaptations illustrate the propensity of the ABH fold to rapidly adapt to catalyze non-canonical reactions essential to organismal survival in challenging ecosystems [7,13,34]. Commercially, HNLs are valued industrially for their ability to catalyze the biosynthesis of cyanohydrins with high stereoselectivity [40].

The methyl ketone (MK) 2-tridecanone, initially isolated from wild tomato (*Lycopersicon hirsutum glabratum*), serves as a chemical defense against herbivory [41]. MK biosynthesis occurs by a two-step enzymatic process that interrupts normal fatty acid elongation. The first step, catalyzed by MKS2, a member of the hotdog fold thioesterase (TE) family, intercepts C12 and C14  $\beta$ -keto acyl-ACP intermediates during fatty acid biosynthesis to release free  $\beta$ -keto fatty acids [42].

The second step in MK production was unexpected given the assumed reactivity of  $\beta$ -keto acids to undergo spontaneous decarboxylation. Instead, the released  $\beta$ -keto fatty acids are generally stable, and decarboxylation is catalyzed by the ABH family member, MKS1 [8–10]. Surprisingly, MKS1’s active site lacks a canonical catalytic triad, possessing only a conserved histidine residue,

His243, while the established active site serine and aspartic acid residues are substituted by Ala87 and Asn215, respectively. In fact, an unexpected and catalytically essential threonine residue, Thr18, is positioned next to His243 and trapped substrate analogs, providing the necessary hydrogen bonding architecture for the efficient decarboxylation of bound  $\beta$ -keto acids (Figure 3) [8]. Alanine substitutions of Thr18 or His243 severely reduce MKS1 catalytic activity. Attempts at converting MKS1 to an active thioesterase via homology-based mutations (Ala87Ser and Asn215Asp mutations) proved unsuccessful, indicating that additional features of MKS1 topological evolution drove its positive selection as a key catalytic component in defensive MK production in plants [8].

Polyneuridine aldehyde esterase (PNAE) plays a key role in the biosynthesis and chemical diversification of monoterpene indole alkaloids of the sarpagine/ajmaline family of plant alkaloids [43]. Well-known compounds from this class of molecules include sarpagine, ajmaline (anti-arrhythmia) and raumacline [43,44]. Additionally, these compounds serve in plant defense largely as insecticides [45]. PNAE resides at a key branch point in the production of downstream ajmaline/sarpagine C9 terpene indole alkaloids by converting polyneuridine aldehyde into 16-epivellosimine, CO<sub>2</sub>, and methanol [11,43,46]. PNAE retains the canonical catalytic triad, but has diverged mechanistically from traditional ABHs to catalyze a bifunctional reaction. It first utilizes the conventional esterase mechanism, releasing methanol, but then upon water-mediated hydrolysis of the covalent Ser87-substrate ester adduct, the enzyme catalyzes decarboxylation of the released intermediate, forming the final product

16-epivellosimine (Figure 3) [11].

The above examples indicate that the plant ABH enzyme family, including HNL, MKS1 and PNAE, catalyze a wide variety of chemical reactions to create structurally diverse classes of primary and specialized metabolites often in restricted plant taxa. Given their key roles in primary and specialized metabolism, hormone regulation and signaling, and defense, ABHs have evolved wide-ranging functional versatility during land plant evolution.

### **ABH fold recruitment as receptors in plants**

Plant hormone metabolism, temporal and spatial regulation of biosynthesis and transport, and signaling through receptors, are all critical topics of research in plant biology. In the past 10 years, there has been substantial progress in identifying and structurally characterizing the receptors for the currently identified plant hormones [19,47–50]. Additionally, plant hormones of the strigolactone family and hormone-like karrikins derived from burnt plant tissue, have been extensively characterized for their synthesis and signaling capacities [17,51]. Many of these newly elucidated hormone signaling pathways regulate gene expression by forming SCF E3 ubiquitin ligase complexes that degrade downstream transcriptional repressors, thereby activating hormone-specific suites of genes (Figures 4 and 5). An interesting facet to this rapidly developing field is the evolutionary selection and adaptation of the ABH fold as hormone–small molecule receptors in the gibberellin, strigolactone, and karrikin

signaling pathways. The gibberellin receptor in *Oryza sativa*, gibberellin insensitive dwarf 1 (GID1), is an ABH that exhibits structural similarity to the hormone sensitive lipases and is a member of the plant carboxylesterase family in the ESTHER database [39,52]. Additionally, the karrikin and strigolactone receptors, KAI2 and DAD2/D14, respectively, are ABH folds belonging to the RsbQ-like family of  $\alpha\beta$ -hydrolase folds [14,15,39,53]. These three receptor families provide an evolutionary glimpse into the selection of a wide-spread protein fold re-appropriated in the green plant lineage to recognize and transduce signals from small molecules to regulate growth and development in a myriad of challenging plant ecosystems.

### **Gibberellin receptor, a catalytically inactive hormone receptor**

Gibberellic acids (GAs), or gibberellins, are an extraordinarily diverse class of diterpenoid-derived molecules that play key roles in the regulation of seed germination, plant growth, and flowering [18,54]. GA biosynthesis and regulation have been previously reviewed [18,54,55]. GA activated gene pathways are repressed by the GRAS family transcriptional repressors known as DELLA proteins, named for the conserved DELLA sequence residing on their N-terminal domains. This DELLA sequence is essential for hormone recognition by the GID1 receptor. Recent structural studies have shed light on the substrate recognition and signal transduction mechanisms of the GID1 receptor [19,56–58]. Structure and sequence comparisons demonstrate that GID1 is an ABH family

member with structural similarity but relatively little sequence identity to the hormone sensitive lipases [39,52]. Interestingly, GID1 lacks a catalytic histidine residue that is part of the canonical ABH family catalytic triad. Moreover, GID1 does not appear to possess catalytic activity, suggesting that it likely acts only as a receptor with no general biosynthetic or metabolic function [19,52,59].

The GID1 structure from *Oryza sativa* in complex with GA4, a physiologically active GA (Figure 1), and the ternary complex of GID1-GA3-GAI (DELLA protein) from *A. thaliana* provide atomic resolution insights into the GA-mediated mechanism of hormone recognition and signal transduction (Figure 4) [19,56,59]. Based on difficulties in crystallizing the GID1 *apo* structure and proteolysis studies, researchers concluded that the N-terminal region is disordered until the receptor binds its GA substrate [58,60]. Unfortunately, no structures are available for *apo* GID1, as attempts to crystallize the protein in the absence of its ligand were unsuccessful [19]. Upon GA binding, the apparently disordered N-terminal domain undergoes a conformational transition to an ordered state, wrapping over the GA molecule and completing the top and back wall of the GA binding pocket (Figure 4B). Interestingly, GA3/GA4 binding to GID1 is enhanced by the coordination of a GA carboxylate moiety by GID1's predicted oxyanion hole. Notably, mutation to the residues conforming to a canonical ABH oxyanion hole significantly decrease GA binding affinity [59]. Solvent exposed hydrophobic residues on the GID1 N-terminal domain serve as the recognition site for DELLA proteins. The interface between GID1 and DELLA buries  $\sim 2,600 \text{ \AA}^2$  and is largely composed of hydrophobic interactions between

GID1's N-terminal region and the conserved DELLA and LExLE motifs of GAI. Upon complex formation, GID1-GAI recruits an SCF E3 ubiquitin ligase complex through the FBOX protein SLY1. SCF E3 then polyubiquitylates DELLA, marking it for degradation by the 26S proteasome (Figure 5) [55,61]. Destruction of DELLA GRAS family transcriptional repressors then relieves the negative regulation of GA-controlled gene expression.

### **Strigolactone and karrikin receptors KAI2 and D14**

Strigolactones and karrikins are recently discovered butenolide-containing plant signaling molecules that have garnered much attention in the plant community [62–64]. Strigolactones, initially discovered for their ability to induce germination of the parasitic plant *Striga hermonthica*, serve as endogenous plant hormones that control shoot branching and as excreted signaling molecules to mediate symbiosis with arbuscular mycorrhizal fungi [51,53,65]. Karrikins are smoke derived compounds shown to stimulate seed germination after fires [15,53,64,66]. Interestingly, karrikins do not appear to be synthesized in plants, but form during the burning of plant tissue. Most notably, they are assumed to mimic a yet to be identified endogenous plant signaling molecule [67,68]. The biosynthesis, formation, and signaling pathways of strigolactones and karrikins have been reviewed previously [62–64,69].

Early studies in *Petunia hybrida* (*Ph*), *Arabidopsis thaliana* (*At*), and *Oryza sativa* (*Os*) identified the strigolactone receptor by way of mutations that resulted

in dwarfed plants with highly branched phenotypes classified as decreased apical dominance (DAD), more axillary growth (MAX), or various dwarf (D) phenotypes, respectively. Similarly, mutations that resulted in karrikin insensitivity (KAI) and light hyposensitivity in *Arabidopsis* led to the discovery of the karrikin receptor KAI2 [62,64]. With regard to the strigolactone and karrikin receptors, D14/DAD2 and KAI2, respectively, they exhibit high sequence identity (~50%, see sequence alignment in Figure 5B), and appear to converge on the same FBOX protein signaling partner, MAX2/D3. However, they are differentially regulated by distinct small molecules resulting in non-overlapping plant growth responses. The latter roles are most clearly discernable by disparate phenotypic effects in plants lacking the respective D14/DAD2 or KAI2 receptors. [70–72]

While KAI2 responds to the exogenous presence of smoke-derived karrikins, the unique phenotypes of KAI2 knock-out plants suggests KAI2 recognizes an endogenous plant molecule possibly emergent during early plant evolution [71,73]. Recently, structures of these ABHs provided ligand-dependent architectural transitions and catalytic hypotheses for these ABH folds possessing canonical Ser/His/Asp catalytic triads (Figure 5C). Notably, this includes a conserved, four-helical V-shaped lid domain important for hormone binding/recognition and signal transduction mediated by protein-protein interactions (Figure 5D) [14–16,74]. Comparison of the two receptors shows a smaller binding pocket for KAI2 than D14, but overall the structures superimpose with an RMSD of ~1.3 Å.

While both receptors retain catalytic activity for the hydrolysis of reactive

and non-physiological substrates, namely *p*-nitrophenylphosphate (PNP) esters, a common hydrolase assay, only D14/DAD2 catalyzes hydrolysis of the synthetic strigolactone GR24, albeit with significantly compromised turnover [17]. Neither KAI2 nor D14 appear to modify karrikins, although a futile cycle of catalytic ring opening and closing is possible [70]. It is interesting to note that AtKAI2 responded to racemic mixtures of GR24 but not to an enantiomer of GR24, specifically GR24<sup>5DS</sup>. GR24<sup>5DS</sup> mimics the naturally biosynthesized enantiomer of strigolactones and is specifically turned over by D14/DAD2 ABHs [70]. Biochemically, AtKAI2 catalyzes hydrolysis of the non-natural enantiomeric analog GR24<sup>ent5DS</sup>, suggesting the endogenous ligand of KAI2 is likely to possess a butenolide-like moiety susceptible to turnover by the catalytic triad of KAI2 [72].

Yeast two-hybrid studies indicate that *Petunia hybrida* DAD2 binds PhMAX2, an SCF E3 ligase FBOX protein, in the presence of strigolactones. Interestingly, mutation of the active site Ser96 to Ala in DAD2 abolishes detectable interactions between DAD2 and PhMAX2A, indicating that the coupling of hormone recognition and hydrolysis likely plays a central role in mediating strigolactone-dependent signal transduction in the D14/DAD2 ABH fold subfamily [14]. It is important to note that strigolactone hydrolysis products themselves do not appear to possess *in vivo* activity [14]. Despite the noted lack of *in vivo* activity, Nakumara *et al.* obtained a structure of D14 complexed with a hydrolyzed product of strigolactone, D-OH. They also found that extremely high concentrations of D-OH (at 50  $\mu$ M) could slightly rescue the strigolactone

biosynthetic mutant phenotype. They proposed D-OH bound D14 is important for signal transduction, suggesting that hydrolysis of full length strigolactones is important for D14's signal transducing role [75].

The strigolactone and karrikin signaling pathways are reminiscent of those of auxin, jasmonic acid (JA), and GAs, where receptors are FBOX proteins (auxin and JA) or bind to FBOX proteins (GA) in SCF E3 ubiquitin ligase complexes (Figure 5A) to regulate the degradation of downstream transcriptional repressors. Interestingly, D14/DAD2 and KAI2 appear to share the same E3 ligase target FBOX protein, D3/MAX2. Nevertheless, ongoing studies are needed to mechanistically elucidate how these two receptors independently regulate signals downstream of D3/MAX2. Recent work in *Oryza sativa* has identified that activation of D14 by strigolactones induces the degradation of D53, which was shown to be a repressor of downstream responses to strigolactones [76]. Similarly, work by Stanga *et al.* identified SMAX1, a D53 homologue in *Arabidopsis*, as a KAI2 signal regulator [77].

Work elucidating the underlying mechanism of strigolactone signaling indicates that D14 can interact with MAX2/D3 or D53 independently of one another in the presence of strigolactones. While the exact sequence of molecular events accompanying ligand recognition, catalysis, protein conformational changes, and formation of protein-protein complexes is still a subject of debate, D14 appears to become more thermally unstable in the presence of strigolactones [14]. Interestingly, strigolactone-dependent interaction with D3 has also been reported to destabilize D14 [74]. To the best of our knowledge,

regulation of D14/DAD2 and KAI2 catalytic activity through protein-protein interactions remains unresolved and would represent an unprecedented function of ABH fold enzymes. Additionally, how the timing and chemical structures accompanying the complex cycle of ABH hydrolytic mechanisms modulates signaling and signal termination through D14/DAD2 and KAI2s is currently a subject of intense debate. The integration of structural biology with genetics, chemistry, and biochemistry will very likely be key to unraveling the events accompanying ligand recognition, catalysis, signaling, and evolution in the strigolactone/karrikin family of ABH receptors.

## Conclusion

The evolution and continuing expansion of protein folds possessing diverse chemistries associated with ligand recognition and catalysis has been an essential feature of the success of sessile plant colonization of the terrestrial earth. This is particularly notable with regard to the diversity of forms and mechanisms of chemical adaptation to some of the most challenging biotic and abiotic features of global ecosystems. In addition to the already broad spectrum of ABH catalytic reactions, recent work on two soil dwelling bacteria in contact with plant roots uncovered cofactor independent oxygenases unexpectedly possessing the ABH fold, namely *Arthrobacter nitroguajacolicus* Rü61a 1-H-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase (HOD) and *Pseudomonas putida* 33/1 1-H-3-hydroxy-4-oxoquinoline 2,4-dioxygenase (QDO). Both catalyze the

dioxygenation of heteroaromatic rings, however they employ a nucleophile/histidine/acid triad in which the aspartic acid is transmigrated to the end of strand  $\beta$ 6 from its standard position at the end of strand  $\beta$ 7 (Figure 6) [12]. Research on ABH fold proteins in green plants has so far uncovered notable examples of the kind of catalytic diversity represented by HOD and QDO and highly conserved three dimensional structures (Figures 1 and 6).

A cursory survey of the *Arabidopsis* genome alone reveals the presence of well-over 638 genes likely encoding ABH folds, representing ~2.3% of the ~27,379 annotated protein coding genes, nearly all of which have yet to be studied [78,79]. As in other organisms, the ABHs will continue to provide a treasure trove of proteins likely to encode unanticipated catalytic and/or receptor functions. Much work remains in order to characterize the full breadth of plant ABH biochemical activities and biological functions across widely divergent taxa of plants. Given the sessile nature of plants and their dependence on specialized metabolism and small molecule signaling for survival and overall fitness in extreme environments, plant ABH research will provide a greater understanding of the adaptive evolution of the ABH fold with likely translational applications to sustainable agriculture, ecosystem revitalization, and global health.

Finally, throughout this review we have described ABH superfamily members assuming that, since they all share highly similar core three dimensional architectures and fold topologies, they also trace their ancestry back to a common aboriginal ABH gene. While it is convenient to infer that all differences among the ABH family are ultimately the result of divergent evolution

from a single ABH ancestral fold, this is not necessarily the only explanation, given the extreme breadth of sequence space sampled by ABH family members across all three domains of life. Indeed, the vast degree of sequence, biochemical, and functional diversity encoded by the ABH fold superfamily is a testament to the relative disconnection between fold/biological function/biochemical activity and nucleotide sequence. Put another way, commonality of fold is neither a proxy for related primary structures nor indication of shared ancestry.

This being the case, it is an intriguing possibility that the modern members of the ABH fold family trace their genetic ancestry back to several, independent ancestral starting points. In support of this notion, remarkable advances in computational protein design approaches, including the Rosetta software suite, have established that protein folds can be accurately designed “from scratch” without reliance on a natural protein sequence to serve as their common ancestor [80,81]. Whether we will be able to infer with statistical confidence the likelihood of such events, and, if so, what would be the number of such starting points are provocative questions to consider in contemporary studies of protein/enzyme design and evolution.

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## Figure Legends

Figure 1: **Structural overview of ABH proteins in green plant lineage.** The 2D structure format of alpha/beta hydrolases is indicated in the center of the figure. N-terminal regions (orange) and inserted lid domains (blue) correspond the 3D structures surrounding the 2D outline. The surrounding ABH structures are intended to demonstrate the diversity of currently solved structures from the green plant lineage. Some ABHs may not contain complete catalytic triads, therefore their active site region along with their catalytic residues are shown with their co-crystallized substrates. The ligand in each structure is represented as dots and colored by atom with Carbon-bright orange, Oxygen-red, and Nitrogen-blue. A close-up of the active site is shown below each, oriented to best view the ligand and catalytic/relevant residues, rather than to remain in the same orientation as the cartoon full view. Chemical structures of the biologically relevant ligands/substrates are also shown. PDB codes are as follows: SABP2 (1XKL), HNL(1SCQ), MKS1(3STU), PNAE(3GZJ), Epoxide Hydrolase (2CJP), KAI2 (3JKM), D14 (5DJ5), GID1(3EBL).

Figure 2: **Maximum Parsimony Tree showing all identifiable ABH-domain encoding genes from *A. thaliana*.** These sequences were collected from the Interpro database (alpha/beta hydrolase fold: IPR029058, [79]). Phylogeny inferred using the Jones-Taylor-Thornton model and all sites with gaps were included. Protein sequences that have been reviewed and entered into the Swiss-Prot have been assigned an Ordered Gene Locus name, while proteins that have not been verified remain in the TrEMBL, have not been assigned an

Ordered Gene Locus, and are labeled in the tree based on their UniProtKB accession code (Red dots). Proteins that are splice isoforms are identified with -1,-2,-3 etc. after the ordered gene locus. Sequence alignments and tree calculation were conducted using MEGA version 5. The alignment file is available as supplementary data, ABH\_Superfamily\_Arabidopsis.meg [82].

**Figure 3: Proposed mechanism of ABHs in four different plant alpha/beta hydrolases.** Figure highlights the catalytic diversity present in plant alpha/beta hydrolases. SABP2 serves as the canonical esterase mechanism, while hydroxynitrile lyase (HNL), polyneuridine aldehyde esterase (PNAE), and methyl ketone synthase (MKS1) serve as non-canonical alpha/beta hydrolase reaction mechanisms that have evolved in plants.

**Figure 4: Signal transduction by the Gibberellin receptor (GID1)** A) Proposed Apo GID1 structure (3EBL with ligand deleted) of flexible N-terminal lid region (Gold) from OsGID1 before becoming ordered due to substrate binding (PDB 3EBL). B) Crystal structure of OsGID1A in complex with gibberellin (GA4) covered by N-terminal lid region. Solvent exposed hydrophobic residues on the N-terminal lid are shown in stick (PDB 3EBL). C) Complex formed between AtGID1 and the helical N-terminal domain from DELLA (red) of GAI. Residues involved with the recognition and interaction between GID1 and GAI are shown as stick and colors correspond to their respective structures (red, DELLA; Gray/Gold, GID1) (PDB 2ZSI). D) The regulation of gibberellin hormone signaling by DELLA proteins and the GID1 receptor. Upon binding GA, the N-terminal region orders itself over the GA binding site, providing an interaction site for

DELLA proteins. GID1-DELLA complex is then recruited to an SCF(SKP1-like Cul1 F-box) E3 ubiquitin-ligase complex, which is comprised of Cullin, the RING-H2 protein RBX1, a ubiquitin conjugating enzyme E2, and an SKP1-like protein that acts as a substrate adaptor to recruit specific FBOX proteins. FBOX proteins are named for their 50 amino acid FBOX domain and are responsible for recruiting E3 ligase substrates destined for 26s proteasomal degradation through polyubiquitylation.[61] After recruitment of the GID1-DELLA complex to the SCF complex by SLY1/SNE, DELLA proteins are polyubiquitylated and destroyed by the 26s proteasome, releasing GA signaling inhibition, allowing transcription of genes under GA control.

**Figure 5: Structural and functional homology of Strigolactone and Karrikin receptors.** A) Proposed signaling pathway for D14/DAD2. D14/DAD2 binds strigolactone and is recruited to the SCF E3 ligase associated FBOX protein, MAX2, in order to form a complex between DAD2/D14, MAX2, and D53. SCF(SKP1-like Cul1 F-box) E3 ubiquitin-ligase complex are comprised of Cullin, the RING-H2 protein RBX1, a ubiquitin conjugating enzyme E2, and an SKP1-like protein that acts as a substrate adaptor to recruit specific FBOX proteins. FBOX proteins are named for their 50 amino acid FBOX domain, originally identified in the mammalian cyclin F protein, and are responsible for recruiting E3 ligase substrates that undergo 26s proteasomal degradation through polyubiquitylation.[61] Upon recruitment by MAX2, D53 is then polyubiquitylated and destroyed through the 26s proteasome pathway, thereby removing inhibition of strigolactone responses. The underlying importance of

strigolactone hydrolysis and signal transduction is still not well understood and is therefore left open to interpretation. The above figure does not take into account that D14/DAD2 can independently bind with D53 or MAX2/D3 in the presence of strigolactone, which may indicate that D14/DAD2 interacts with D53 before MAX2/D3. B) Sequence alignment of *A. thaliana* KAI2 (PDB: 4jym) and *O. sativa* D14 (PDB: 5dj5), with the lid domains indicated with a blue line. Identical and similar positions are indicated in black and grey boxes, respectively. C) Structural topology of KAI2 and D14 with the lid domains indicated in blue helices and the core domain in grey. The catalytic triad residues are labeled; the positions are numbered in the order, KAI2/D14. D) X-ray crystal structures of KAI2 and D14 are shown as cartoon diagrams and superimposed. Lid domains are colored in dark blue (KAI2) and light blue (D14), core domains in dark grey (KAI2) and light grey (D14). Zoom in picture of the active site shows the catalytic triads and ligands Karrikin (bright orange) and the strigolactone analog, G24 (light orange).

Figure 6: **Superposition of KAI2 and QDO shows structural conservation and functional diversity.** A) Sequence-independent structural superposition of *A. thaliana* KAI2 (PDB: 3JKM) and *Pseudomonas putida* 1H-3-hydroxy-4-oxoquinoline 2,4-dioxygenase (QDO, PDB: 3IBT), with core domains shown in dark and light grey, and the lid domains shown in dark and light blue, respectively. Karrikin is in bright orange sticks with dots in the KAI2 active site. Zoom in view of the active site highlights, that despite the distance in primary structure, the two enzymes position their catalytic triad aspartates in the same location, enabling efficient H-bonding with their respective catalytic histidines. B)

Topology diagrams showing various differences in the core domains of both enzymes and the differences in catalytic aspartate placement within each structure.

## Figures and Legends

### Figure 1:

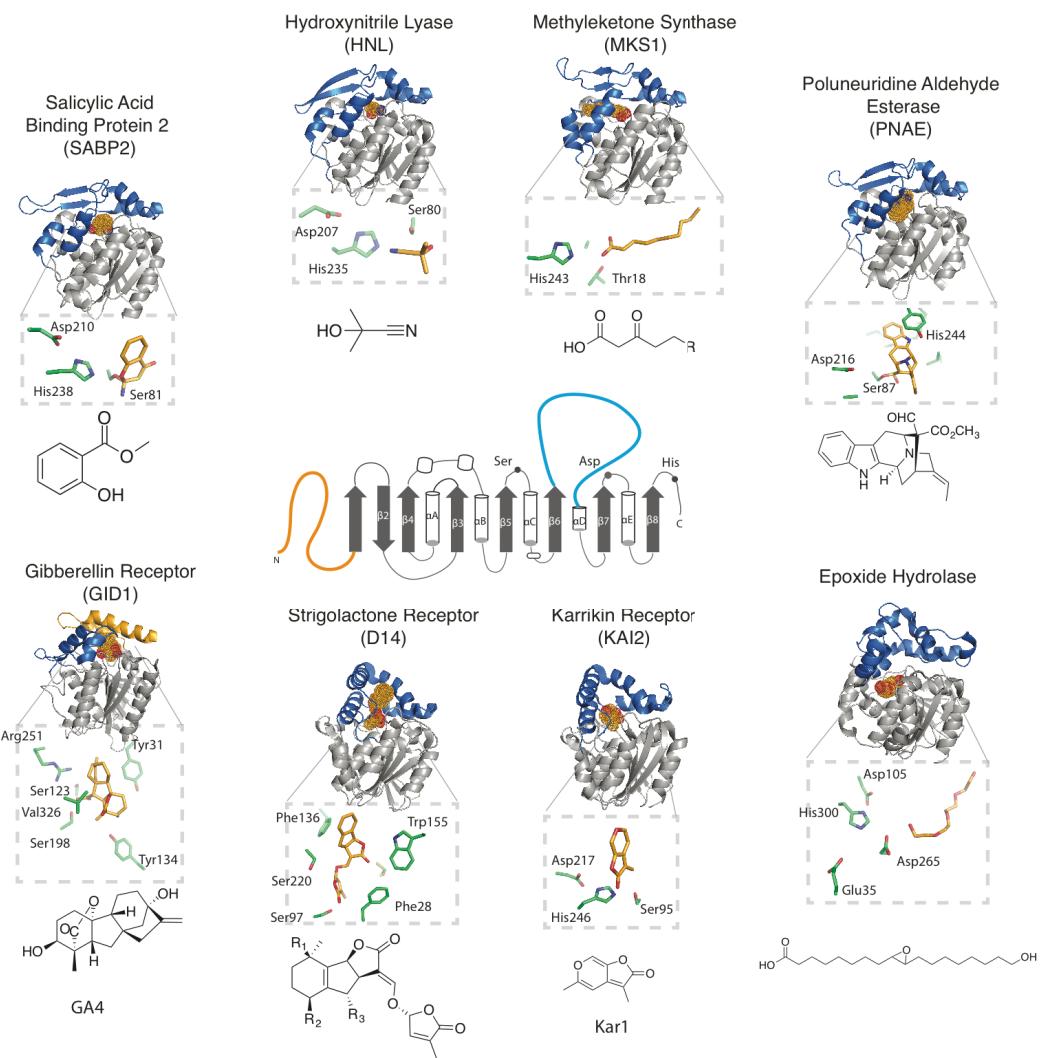


Figure 1: The 2D structure format of alpha/beta hydrolases is indicated in the center of the figure. N-terminal regions (orange) and inserted lid domains (blue) correspond the 3D structures surrounding the 2D outline. The surrounding ABH structures are intended to demonstrate the diversity of currently solved structures from the green plant lineage. Some ABHs may not contain complete catalytic triads, therefore their active site region along with their catalytic residues are shown with their co-crystallized substrates. The ligand in each structure is represented as dots and colored by atom with Carbon-bright orange, Oxygen-red, and Nitrogen-blue. A close-up of the active site is shown below each, oriented to best view the ligand and catalytic/relevant residues, rather than to remain in the same orientation as the cartoon full view. Chemical structures of the biologically relevant ligands/substrates are also shown. PDB codes are as follows: SABP2 (1XKL), HNL(1SCQ), MKS1(3STU), PNAE(3GZJ), Epoxide Hydrolase (2CJP), KAI2 (3JKM), D14 (5DJ5), GID1(3EBL).

Figure 2:

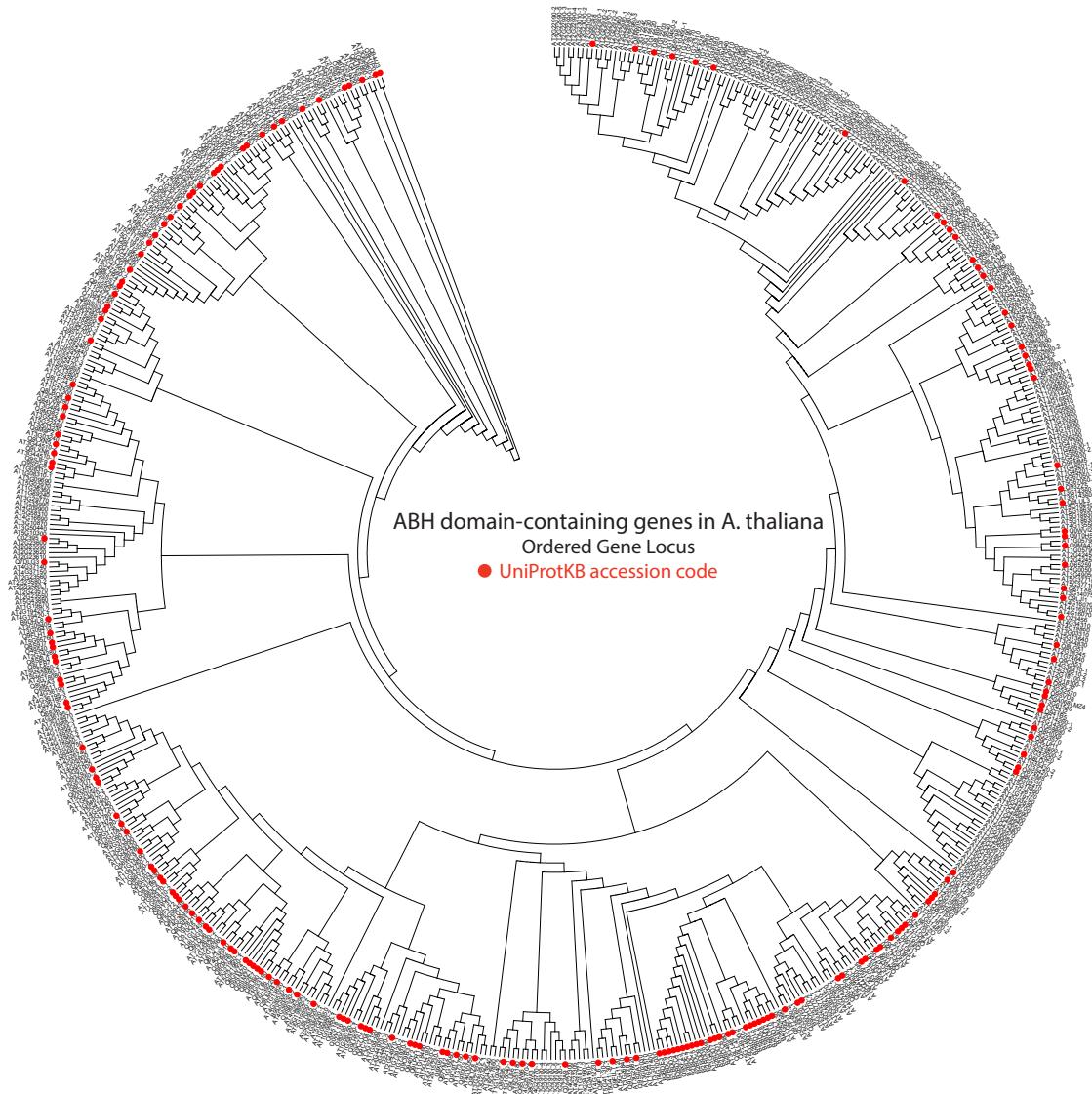
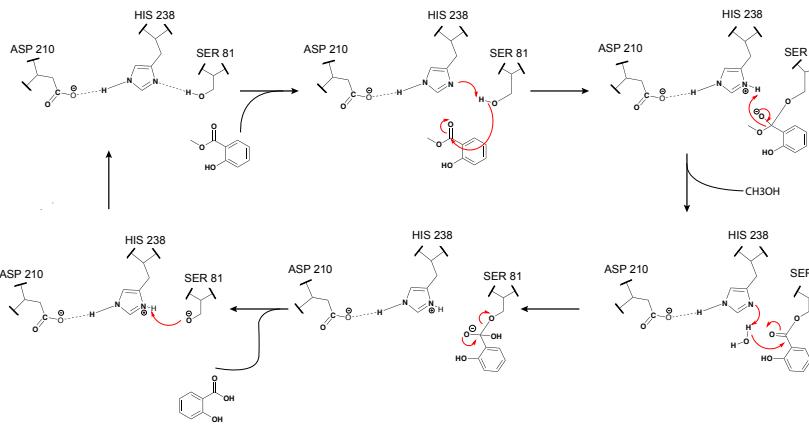


Figure 2: Maximum Parsimony Tree showing all identifiable ABH-domain containing genes from *A. thaliana*, collected using the Interpro database (Alpha/Beta hydrolase fold: IPR029058, [80]). Phylogeny inferred using the Jones-Taylor-Thornton model and all sites with gaps were included. Protein sequences that have been reviewed and entered into the Swiss-Prot have been assigned an Ordered Gene Locus name, while proteins that have not been verified remain in the TrEMBL,

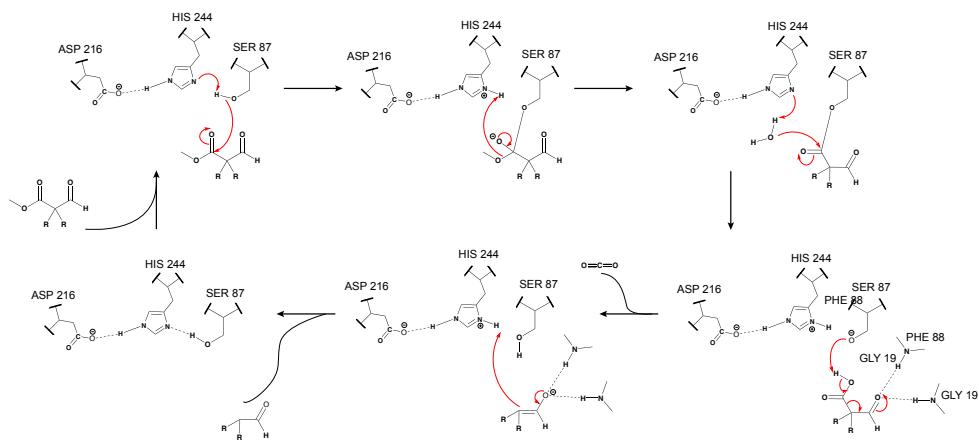
have not been assigned an Ordered Gene Locus, and are labeled in the tree based on their UniProtKB accession code (Red dots). Proteins that are splice forms are identified with -1,-2,-3 etc. after the ordered gene locus. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5. [20]

Figure 3:

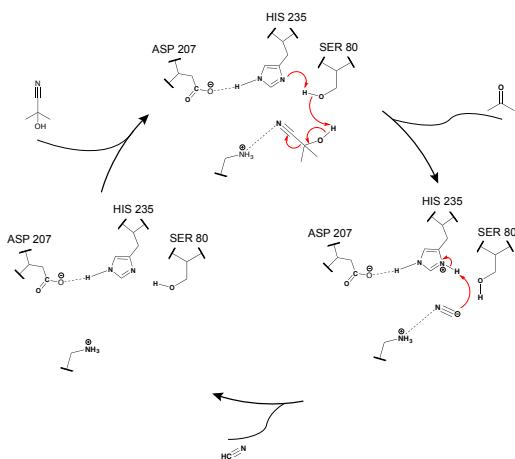
## SABP2



## PNAE



## HNL



## MKS1

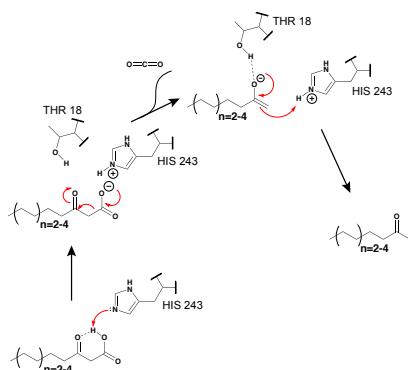


Figure 3: Proposed mechanism of alpha-beta hydrolases in four different plant alpha/beta hydrolases, highlighting the catalytic diversity present in plant alpha/beta hydrolases. SABP2

serves as the canonical esterase mechanism, while hydroxynitrile lyase (HNL), polyneuridine aldehyde esterase (PNAE), and methyl ketone synthase (MKS1) serve as non-canonical alpha/beta hydrolase reaction mechanisms that have evolved in plants.

Figure 4:

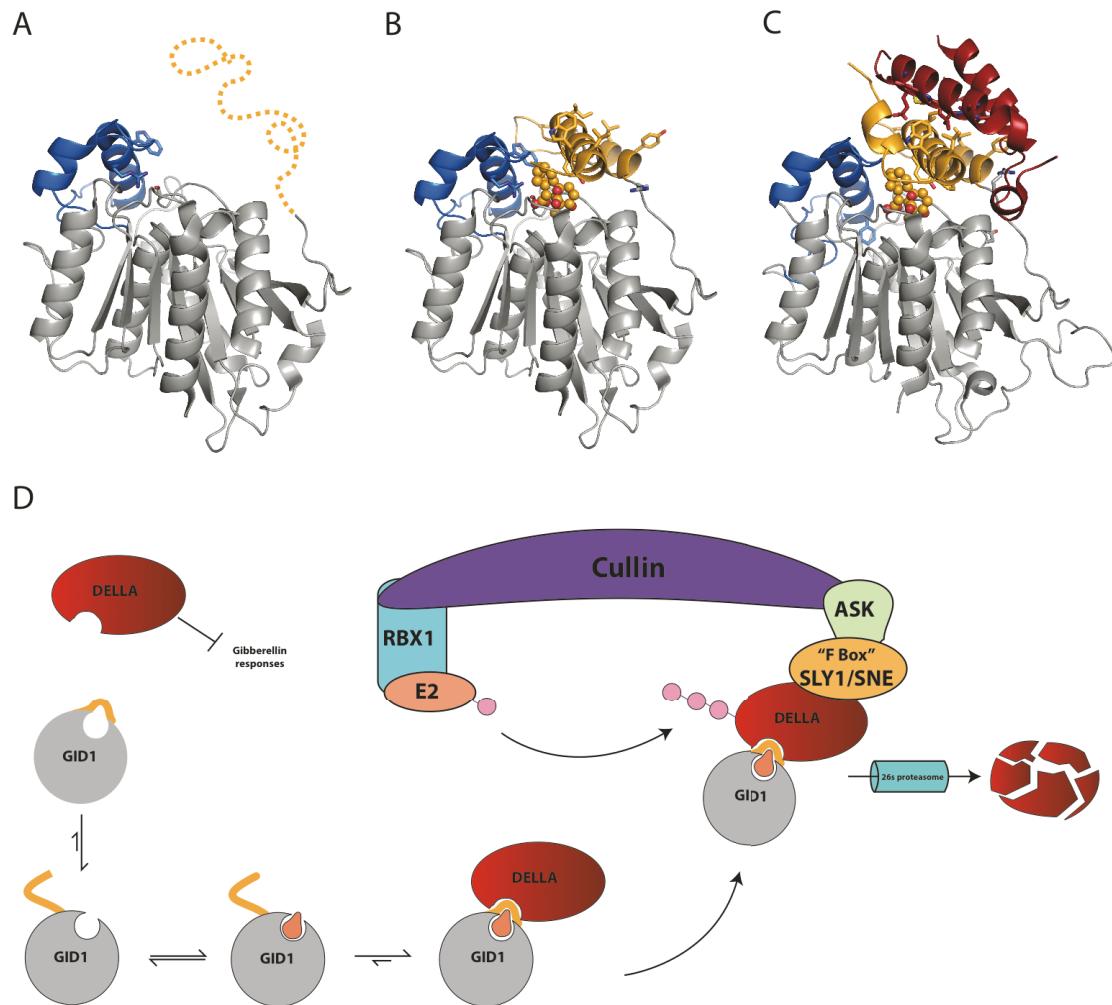


Figure 4: Three structures of the gibberellin receptor (GID1) A) Proposed Apo GID1 structure (3EBL with ligand deleted) of flexible N-terminal lid region (Gold) from OsGID1 before becoming ordered due to substrate binding (PDB 3EBL). B) Crystal structure of OsGID1A in complex with gibberellin (GA4) covered by N-terminal lid region. Solvent exposed hydrophobic residues on the N-terminal lid are shown in stick (PDB 3EBL). C) Complex formed between AtGID1 and the helical N-terminal domain from DELLA (red) of GAI. Residues involved with the recognition and interaction between GID1 and GAI are shown as stick and colors correspond to their respective structures (red, DELLA; Gray/Gold, GID1) (PDB 2ZSI). D) The regulation of gibberellin hormone signaling by DELLA proteins and the GID1 receptor. Upon binding GA, the N-terminal region orders itself over the GA binding site, providing an interaction site for DELLA proteins. GID1-DELLA complex is then recruited to an SCF(SKP1-like Cul1 F-box) E3 ubiquitin-ligase complex, which is comprised of Cullin, the RING-H2 protein RBX1, a ubiquitin conjugating enzyme E2, and an SKP1-like protein that acts as a substrate adaptor to recruit specific FBOX proteins. FBOX proteins are named for their 50 amino acid FBOX domain and are responsible for recruiting E3 ligase substrates destined for 26s proteasomal degradation through polyubiquitylation.[61] After recruitment of the GID1-DELLA complex to the SCF complex by SLY1/SNE, DELLA proteins are

polyubiquitinylated and destroyed by the 26s proteasome, releasing GA signaling inhibition, allowing transcription of genes under GA control.

Figure 5:

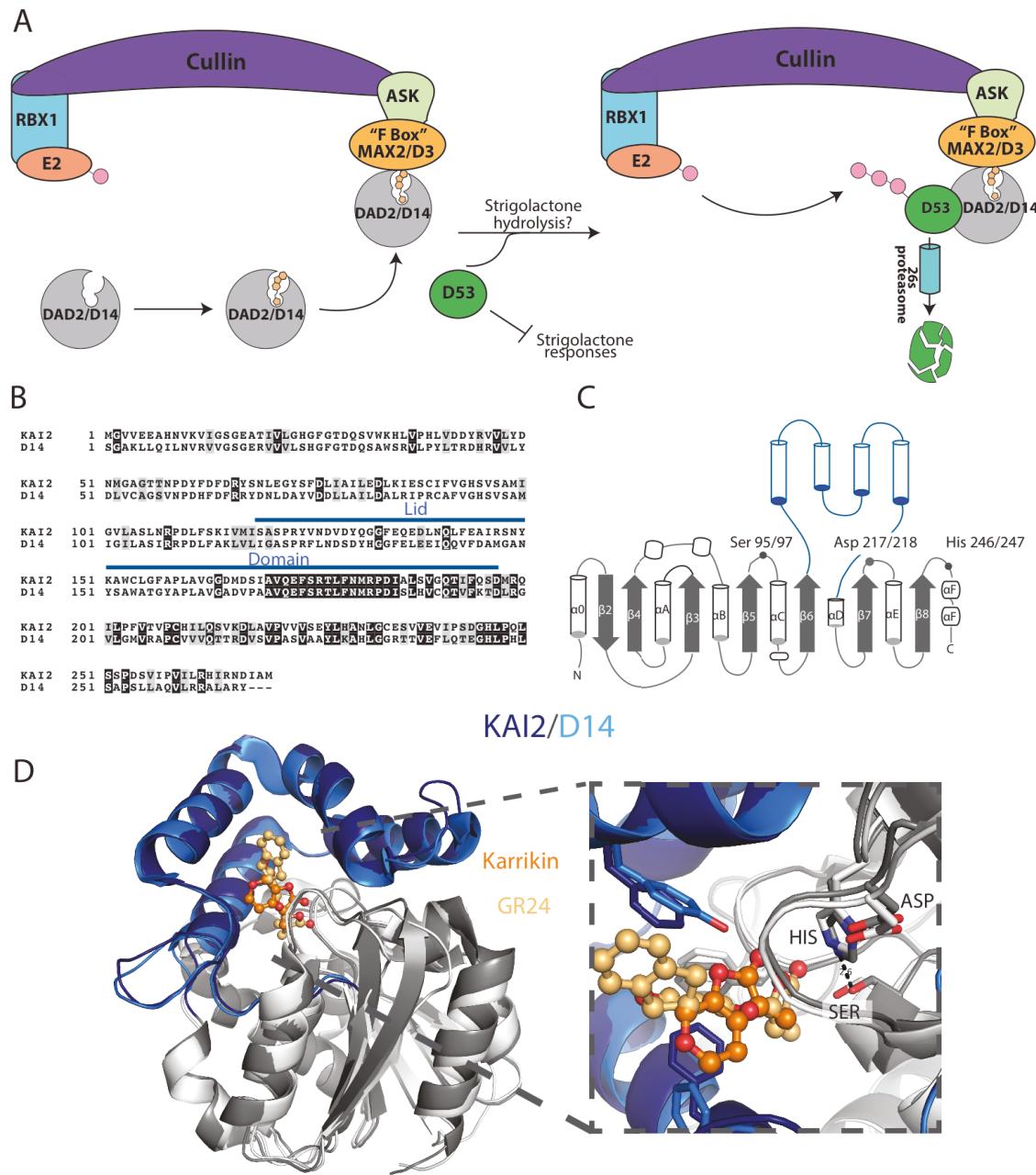


Figure 5: A) Proposed signaling pathway for D14/DAD2. D14/DAD2 binds strigolactone and is recruited to the SCF E3 ligase associated FBOX protein, MAX2, in order to form a complex between DAD2/D14, MAX2, and D53. SCF(SKP1-like Cul1 F-box) E3 ubiquitin-ligase complex are comprised of Cullin, the RING-H2 protein RBX1, a ubiquitin conjugating enzyme E2, and an SKP1-like protein that acts as a substrate adaptor to recruit specific FBOX proteins. FBOX proteins are named for their 50 amino acid FBOX domain, originally identified in the mammalian cyclin F protein, and are responsible for recruiting E3 ligase substrates that undergo 26s

proteasomal degradation through polyubiquitylation.[61] Upon recruitment by MAX2, D53 is then polyubiquitylated and destroyed through the 26s proteasome pathway, thereby removing inhibition of strigolactone responses. The underlying importance of strigolactone hydrolysis and signal transduction is still not well understood and is therefore left open to interpretation. The above figure does not take into account that D14/DAD2 can independently bind with D53 or MAX2/D3 in the presence of strigolactone, which may indicate that D14/DAD2 interacts with D53 before MAX2/D3. B) Sequence alignment of *A. thaliana* Kai2 (PDB: 4jym) and *O. sativa* D14 (PDB: 5dj5), with the lid domains indicated with a blue line. Identical and similar positions are indicated in black and grey boxes, respectively. C) Structural topology of Kai2 and D14 with the lid domains indicated in blue helices and the core domain in grey. The catalytic triad residues are labeled; the positions are numbered in the order, Kai2/D14. D) X-ray crystal structures of Kai2 and D14 are shown as cartoon diagrams and superimposed. Lid domains are colored in dark blue (Kai2) and light blue (D14), core domains in dark grey (Kai2) and light grey (D14). Zoom in picture of the active site shows the catalytic triads and ligands Karrikin (bright orange) and the strigolactone analog, G24 (light orange).

Figure 6:

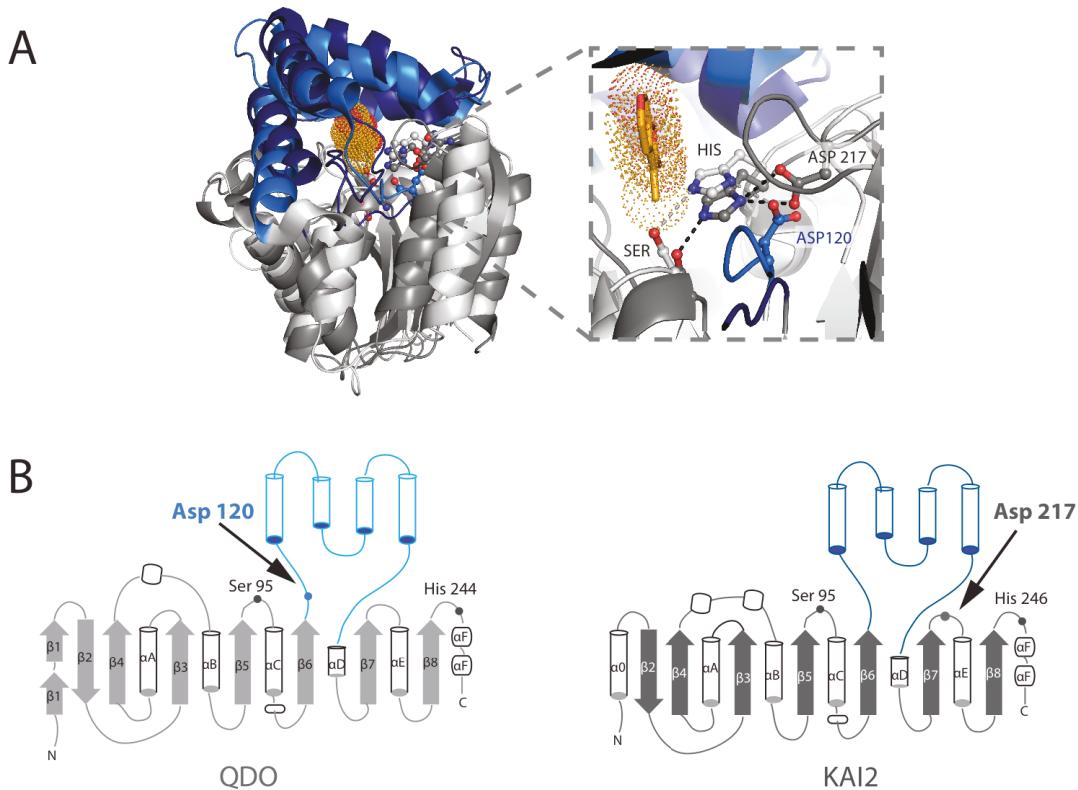


Figure 6: A) Sequence-independent structural superposition of *A. thaliana* Kai2 (PDB: 3JKM) and *Pseudomonas putida* 1H-3-hydroxy-4-oxoquinoline 2,4-dioxygenase (QDO, PDB: 3IBT), with core domains shown in dark and light grey, and the lid domains shown in dark and light blue, respectively. Karrikin is in bright orange sticks with dots in the Kai2 active site. Zoom in of the active site highlights that despite the distance in primary sequence, the two enzymes have their catalytic triad Aspartates in largely the same location, enabling efficient H-bonding with their respective catalytic Histidines. B) Topology diagrams showing various differences in the core domains of both enzymes and the differences in Catalytic Aspartate placement within the structure.