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Absciscic acid (ABA) receptors: light at the end of the tunnel

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Abstract

The plant hormone absciscic acid (ABA) plays a role in several aspects of plant growth and development. Understanding how this hormonal stimulus is sensed and transduced turned out to be one of the major tasks in the field of plant signaling. A series of recent papers proposed several different proteins that could receive the ABA signal and initiate the signaling cascade. The winner appears to be PYR/PYL/RCAR (PYrabactin Resistance/PYrabactin Resistance-Like/Regulatory Component of Absciscic acid Receptor) proteins, as crystal structures were recently published. The crystal structures support the idea that upon ABA binding to a PYR/PYL/RCAR protein, the activity of a phosphatase 2C, with known repressive activity on ABA signaling, is inhibited.

Introduction and context

The first report of this series of disparate communications about absciscic acid (ABA) receptors began when Razem *et al.* [1] claimed that the RNA-binding protein FCA, known to promote flowering, was an ABA receptor. They performed binding experiments using recombinant FCA protein purified from *Escherichia coli* and radiolabeled (+) ABA. The FCA story ended quickly when the Macknight and Day group reported that FCA did not bind ABA [2], leading the authors, Razem *et al.*, to retract their paper [3].

The second protein proposed as an ABA receptor was CHLH (magnesium-chelatase subunit H), an Arabidopsis homolog of a bean ABA-binding protein [4]. These authors showed that Arabidopsis CHLH was able to bind ABA and that RNAi (RNA interference) lines with reduced expression of *CHLH* were insensitive to ABA. However, given the strength of the *chlh* mutant, *chlh* alleles should have been isolated as ABA-insensitive mutants in previous genetic screens but were not. Furthermore, the barley magnesium-chelatase *XanF* did not bind ABA and none of the different *XanF* mutants

showed any altered ABA responses [5]. Nonetheless, it is possible, despite the high amino acid identity of the Arabidopsis and barley CHLH proteins (close to 82%), that only the Arabidopsis CHLH would function as an ABA receptor.

The third protein proposed as an ABA receptor was GCR2, a hypothetical G protein-coupled receptor (GCR) with the expected seven-transmembrane (7TM) domain structure [6]. Liu *et al.* [6] claimed that recombinant GCR2 protein (also prepared in *E. coli*) bound ABA. But other groups showed that GCR2 was not necessary for several ABA responses [7,8] and that it was more similar to bacterial lanthione synthetases [9,10] than to a 7TM protein, as Liu *et al.* [6,10] stated. The main problem of Liu *et al.* was that they used FCA as a positive control in their binding assays, predictably leading the Macknight and Day group to show that GCR2 did not bind ABA either [11]. All of these issues are still under dispute because, despite several studies that failed to confirm the results, there was no formal retraction by Liu *et al.* [6] or any new findings to support GCR2 as a *bona fide* ABA receptor.

Major recent advances

In 2009, two new groups of proteins attempted to gain membership in the ABA receptor club. The first group is comprised of two membrane proteins (GTG1 and GTG2) with nine predicted transmembrane domains [12]. GTG1 and GTG2 both bind ABA, and *gtg1;gtg2* mutant plants showed reduced but not completely abolished ABA responses. The *in vitro* binding assays with GTG1 and GTG2 were performed by expressing them in *E. coli* and using the soluble fraction. The stoichiometry of the binding was very low, such that only 1% of the GTGs purified from *E. coli* were able to bind ABA (Table 1). The authors attributed this insignificant binding to poor protein purification, solubilization, or renaturation. However, as they admitted, physiological environments more representative of those experienced by eukaryotic membrane proteins will be needed to demonstrate that GTG1 and GTG2 are true ABA receptors.

HAB1, *ABI1*, and *ABI2* encode protein phosphatase 2 Cs (PP2Cs) that act as negative regulators of ABA signaling [13]. The second group of candidate ABA receptors is comprised of proteins (pyrabactin resistance/pyrabactin resistance-like/regulatory component of abscisic acid receptor, or PYR/PYL/RCAR) that, through ABA binding, would inhibit the known repressive activity of PP2Cs on ABA signaling. Park *et al.* [14] used an elegant chemical genetic strategy to identify and characterize a family of StAR-related lipid transfer (START) proteins (called PYR1/PYL1-13). They used heteronuclear single-quantum coherence-nuclear magnetic resonance to show that, in the presence of saturating amounts of bioactive ABA, PYR1 binds HAB1 (homology to

ABA-insensitive 1 [ABI1]). The quadruple mutant *pyr1;pyl1;pyl2;pyl4* displayed ABA insensitivity in seed germination and root growth assays [14] and in ABA-regulated stomatal movements [15]. Park *et al.* [14] used recombinant proteins to demonstrate that PYR1 inhibits the phosphatase activity of a PP2C only in the presence of ABA. Another group used a yeast two-hybrid approach for HAB1-interacting proteins and isolated three proteins from this family (PYL5, PYL6, and PYL8) [16]. Ma *et al.* [17] also used a yeast two-hybrid approach for binding partners for ABI2 and identified a START protein (which they named RCAR1). They used isothermal calorimetric analysis to demonstrate that (S)-ABA binds to RCAR1 and ABI2. Park *et al.* [14] suggested that the PYR/PYL/RCAR interactions with PP2Cs are sensitive to protein concentration, implying that (at least in yeast) some of the PYR/PYL/RCARs can interact with PP2Cs in the absence of ABA. However, inhibiting the phosphatase activity of ABI1/2 with RCAR1/PYL9 requires ABA because when Ma *et al.* [17] added greater than 15-fold excess RCAR1/PYL9 to 0.1 mg of ABI1/2, there was no inhibition of the phosphatase activity in the absence of ABA.

Crystal structures of PYR1, PYL1, and PYL2 were recently published [18-22]. In the ligand-free conformation, the receptors expose a pocket that is vacant and that is exclusively occupied later by ABA. Two highly conserved surface loops are located at the pocket entrance. When ABA binds, allosteric changes in the two loops close the pocket, confining ABA inside the pocket and thereby exposing a PP2C domain-binding site on the loops. PP2C binding stabilizes the closed stage, thereby reducing ABA dissociation. This explains why the affinity

Table 1. Biochemical characteristics of the proposed abscisic acid

Protein	Extract used for binding assays	Moles of ABA per moles of protein	Method used for binding	Dissociation constant (K_d), nanomolar	Localization	Structure	Status	Reference
FCA	<i>Escherichia coli</i>	0.72	^3H -ABA	19	Soluble	No	Retracted	[1]
CHLH	Yeast	1.28	^3H -ABA	32	Stroma and envelope membrane	No	Under dispute	[4]
GCR2	<i>E. coli</i>	0.8	^3H -ABA	20.1	7TM domain	No	Under dispute	[6]
GTGs	<i>E. coli</i>	0.01	^3H -ABA	35.8 (GTG1), 41 (GTG2)	Membrane proteins	No	Recently published	[12]
PYR/PYL	<i>E. coli</i>	0.7	Isothermal titration calorimetry	38 (PYL5-HAB1)	Soluble	Yes	Recently published	[14]
RCAR	<i>E. coli</i>	1.08	Isothermal titration calorimetry	64 (RCAR1-ABI2)	Soluble	Yes	Recently published	[17]

7TM, 7 transmembrane; ABA, abscisic acid; ABI, abscisic acid-insensitive protein phosphatase mutant; CHLH, magnesium-chelatase subunit H; FCA, flowering time control protein; GCR2, G protein-coupled receptor 2; GTG, type G protein; HAB1, homology to abscisic acid-insensitive protein phosphatase mutant 1; PYL, pyrabactin resistance-like; PYR, pyrabactin resistance; RCAR, regulatory component of abscisic acid receptor.

of ABA for PYR/PYL/RCARs is increased by more than 10-fold in the presence of PP2Cs. All of the structures show that when ABA binds to PYR/PYL/RCAR, they inhibit the phosphatase activity of PP2C, resulting in full activation of the ABA signaling pathway. Because the PP2C-binding site perfectly coincides with the PP2C active site, the ABA-bound PYR/PYL/RCAR receptors can be considered competitive inhibitors of the PP2C substrates. Melcher *et al.* [18] elegantly supported this hypothesis by showing that increasing concentrations of a PP2C substrate (a SnRK2.6 peptide containing residues 170-180 and a phosphorylated serine at position 175) de-repressed the inhibition of the PP2C activity caused by ABA-bound PYL2.

Future directions

The structures definitely show that PYR/PYL/RCAR proteins are *bona fide* ABA receptors. But what about the other proposed ABA receptors? Is there still room in the ABA signaling pathway for them? To convince the scientific community that their genes are '*bona fide* ABA receptors', these researchers should first demonstrate in a more careful way that their putative receptors truly bind ABA. Given that GCR2, GTGs, and CHLH are membrane proteins, it would be more appropriate to perform ABA-binding assays in a membranous environment, perhaps in a heterologous eukaryotic system that does not have an ABA signaling pathway. Showing that ethylene bound to yeast expressing ETR1 (ethylene receptor 1) [23] and the assays demonstrating that auxin bound to TIRs (transport inhibitor responses) in insect [24] and *Xenopus* [25] cells are examples that should be imitated.

In general, we expect that mutants in presumed receptors should show reproducible biological effects for at least some final hormonal responses. This expectation was met with the mutants in *CHLH*, *GCR2*, *GTG*, and *PYR/PYL/RCAR* in *Arabidopsis*, which showed phenotypes affecting seed germination, lateral root formation, or stomatal responses. On the other hand, *fca-1* mutants had no defects in any typical ABA responses. There is still some dispute about whether *GCR2* and *CHLH* truly bind ABA, but because of the mutant phenotypes, these proteins may act as modulators or transducers of ABA signaling rather than as receptors. It will be a challenge to envision how membrane proteins such as *GCR2* and *GTGs* could interact with the PYR/PYL/RCAR receptors in order to regulate the binding of ABA or modulate its signaling.

Interestingly, structural studies on the auxin [26] and gibberellin (GA) [27] receptors also proposed that hormones stabilize the interactions between their respective receptors and downstream repressors, even

though the types of molecules involved in all of these different hormonal signaling pathways are entirely different. The ABA receptors directly bind ABA, and this is therefore analogous to the GA receptor system, as GA binds directly to its receptor, GA insensitive dwarf 1 (GID1), and ABA binding and GA binding to their receptors both produce allosteric modifications necessary for the interaction with their effectors, PP2Cs and DELLAs, respectively. However, there are distinct mechanistic details; while DELLA binding to GID1 induces a conformational change in the GRAS domain of DELLA proteins, thus facilitating binding to the F-box protein SLY1 that targets the DELLA proteins for degradation, the binding of PP2C directly inhibits the phosphatase activity because the active site of PP2C is also the site that binds to the ABA receptor.

Gene redundancy explains why ABA loss-of-function mutants for single PYR/PYL/RCAR genes were not obtained as Park *et al.* [14] had to build triple or quadruple *pyr/pyl/rcar* mutants to show strong ABA insensitivity. It will be interesting to investigate whether all members of this protein family are redundant or whether any have unique functions or show cell-specific expression patterns. Why were gain-of-function *pyr/pyl/rcar* mutants (i.e., those that would constitutively inhibit PP2C activity, even in the absence of ABA) not isolated? As two loops are in charge of exposing the PP2C-binding site, perhaps mutation at more than one amino acid residue would be necessary to permanently close the lids and recruit PP2C to its inhibitory stage. However, hypermorphic ABA-insensitive mutants have been obtained [28,29]. When a conserved glycine in the active sites of ABI1 (G180), ABI2 (G168), and HAB1 (G246) was mutated to Asp, dominant ABA-insensitive phenotypes were obtained, presumably because the interaction of the mutated PP2Cs with PYR1 was disrupted, thereby eluding the negative regulation of the ABA-PYR/PYL/RCAR complex. However, these mutants also showed reduced phosphatase activity, at least when using heterologous substrates. To add another perspective to the inhibitory role for PP2C, it was recently shown [29] that ABI1^{G180D} was more efficiently sent to the nucleus than was wild-type ABI1, suggesting that the hypermorphic phenotype is at least partly mediated by nuclear localization, despite the reduced dephosphorylating activity of ABI1^{G180D}.

Although historically it used to be thought that plant hormone receptors must be plasma membrane proteins with expected domains for canonical receptors, the receptors for auxin, jasmonic acid, GAs, and now PYR/PYL/RCAR for ABA show that open minds can lead to new discoveries. Nonetheless, in order for the

scientific community to accept and adopt such new discoveries, multiple approaches are needed.

Abbreviations

7TM, seven-transmembrane; ABA, abscisic acid; ABI, abscisic acid-insensitive protein phosphatase mutant; CHLH, magnesium-chelatase subunit H; FCA, flowering time control protein; GA, gibberellin; GCR, G protein-coupled receptor; GID1, gibberellin insensitive dwarf 1; HAB1, homology to abscisic acid-insensitive 1; PP2C, protein phosphatase 2C; PYL, pyrabactin resistance-like; PYR, pyrabactin resistance; RCAR, regulatory component of abscisic acid receptor; START, steroidogenic acute regulatory protein (StAR)-related lipid transfer.

Competing interests

The authors declare that they have no competing interests.

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