PERSPECTIVE

Abscisic acid (ABA) signaling: finding novel components off the beaten track

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Abstract

The sesquiterpene abscisic acid (ABA) is an ancient stress response molecule. In plants, many ABA-dependent processes operate via PYR/PYL/RCAR receptor complexes, but results from several studies have suggested that not all plant responses function through this mechanism. Since the ABA-dependent processes of animals and humans also operate in the absence of such receptors, we hypothesize that plant and animal proteomes harbour proteins with undiscovered ABA-binding sites. We propose that carefully curated amino acid search motifs deduced from the binding sites of experimentally confirmed ABA-binding proteins can identify many more candidates in plant and animal proteomes. Some of these candidates show structural folds that are compatible with ABA-binding. This approach identifies plant candidates including annotated ABA downstream signaling components SnRK2.2 and SnRK2.6, and proteins involved in protein folding and RNA polyadenylation. The identified ABA-binding candidates in the human proteome affect among other processes, immune responses and tumor progression. If these candidates are eventually validated experimentally, it will imply that the regulation and tuning of ABA-dependent processes is considerably more complex than hitherto suspected. It will also help to clarify the role of this conserved signaling molecule in mammals.

Introduction

The discovery of the chemical nature of abscisic acid (ABA) and the ABA-dependent physiological responses in higher plants were first reported over half a century ago (Cornforth et al. 1965a, b; Mittelheuser and van Steveningk 1969).

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Thereafter, an ever growing body of scientific literature (> 10,000 articles in PubMed) has described the stimulus-induced ABA synthesis, as well as the ABA-dependent signaling and physiological responses, at the molecular and systems level (Nakashima and Yamaguchi-Shinozaki 2013; Wang et al. 2015; Chong et al. 2019; Chen et al. 2020; Takahashi et al. 2020; Lin et al. 2021). Moreover, ABA has been reported to regulate cell responses and biological processes, not just in plants but also in mammals and other organisms, across the kingdom of life (Takezawa et al. 2011; Lievens et al. 2017). It is likely that the molecular resolution of ABA-dependent processes employing the well-characterized signaling components, notably the PYR (Pyrabactin resistant), PYL (Pyrabactin-like regulatory components of ABA receptors), PP2C-A (clade A type 2 C protein phosphatases), and SnRK2 (plant specific serine protein kinases with a role in responses to ABA) (Ali et al. 2020; Lin et al. 2021), will continue to be refined. It is equally likely that alternative mechanisms involving hidden ABA-binding sites will be discovered.

The synthesis and signaling of ABA in plants and in different mutualistic and pathogenic systems, as well as the particular role of ABA as a modulator of host immune responses, has been extensively reviewed (Lievens et al. 2017). It is intriguing that ABA is also found in animals, albeit poorly understood (Le Page-Degivry et al. 1986) and, given that animals do not have a canonical PYR/PYL/RCAR signaling system, they may provide us with important clues for the elucidation of alternative ABA-dependent mechanisms and processes.

ABA has been reported in the early metazoans (Porifera) (Zocchi et al. 2001). It was shown that firstly, heat causes ABA production, and secondly, ABA stimulates protein kinase-A (PKA) to activate ADP-ribosyl cyclase. The resulting cADPR generation then causes the release of Ca^{2+} ions. Another report demonstrates that ABA, naturally taken up via the nectar (Lipp 1990) has many beneficial effects in bees (*A. mellifera*) including boosting their innate immune defence leading to an increased tolerance to pesticides (Negri et al. 2015). ABA also enhances cold tolerance in honeybee larvae with a concomitant modulation of gene expression of stress response proteins and proteins diagnostic for metabolic adjustments.

ABA effects have also been investigated in human granulocytes, insulin-producing rat insulinoma cells, and human and murine pancreatic β cells which produce and release ABA and induce a signal cascade that includes G-protein complexes, cAMP, cADP-ribose, and intracellular Ca^{2+}. Perhaps the most significant contribution to our understanding of ABA signaling in animal cells was the discovery of ABA binding to the lanthionine synthetase C-like protein 2 (LANCL2) and its role in granulocytes and rat insulinoma cells (Sturla et al. 2009). It may also not be a coincidence that the animal LANCL protein family shares structural similarities with the prokaryotic lanthionine synthetase component C proteins which are bacterial membrane associated proteins involved in the synthesis of antimicrobial peptides (Bauer et al. 2000). Since it has been suggested that LANCL2 links ABA interactions to anti-inflammatory and anti-diabetic responses, it might inform the development of synthetic ABA agonists (Sturla et al. 2009). ABA transport has also been studied in human erythrocytes and is shown to occur via a Band 3 anion transporter. Once inside the cell, ABA can stimulate ATP release through the LANCL2-mediated activation of an adenylate cyclase (Vigliarolo et al. 2015) and the corresponding cAMP signaling pathway. ABA also affects inflammatory processes and again LANCL2 is a molecular target for ABA with subsequent G-protein linked cAMP accumulation in immune cells (Bassaganya-Riera et al. 2011). Last but not least, ABA has also been linked to tumor dormancy and reactivation (Jung et al. 2021).

In search of hidden ABA-binding proteins

Here we propose that plant and animal proteomes contain proteins that harbour hitherto undiscovered ABA-binding sites and that binding of ABA may specifically modulate their properties (e.g., enzyme activities) thereby modulating biological processes essential for growth, development, and adaptation. This proposition is essentially based on the observation that ABA can specifically bind to components other than the PYR/PYL/RCAR complex and mediate ABA-specific effects. Three of the many examples illustrate this point. Firstly, an affinity-based chemical proteomics approach has led to the identification of mitochondrial adenine nucleotide translocators (ANTs) as ABA-binding in vitro (Kharenko et al. 2011). Subsequent analyses demonstrated that ANT mediated ATP translocation was inhibited by ABA. The results are therefore entirely consistent with a direct role of ABA on mitochondrial ATP translocation across the matrix and a direct and rapid role of ABA in plant energy metabolism. In a later series of experiments, an ABA mimetic probe combined with in vitro binding assays in *Arabidopsis thaliana* identified additional candidate ABA-binding proteins including Rubisco (Galka et al. 2015) thus directly linking ABA to CO_2 photosynthetic metabolism and affecting the pool size of photosynthetic intermediates and carbon metabolism in general (Suzuki et al. 2012). Secondly, a similar affinity-based method employing biotin linkers containing both alkyne and amino groups, a protein crosslinker, and ABA azido probes, that circumvents the reduction in biological activities associated with direct attachment of large functional groups to ABA, has identified a thioredoxin from *A. thaliana* (AtTrxh3) as an ABA-binding protein. However, in vitro data showed no influence of ABA on the thioredoxin and chaperonin activities of AtTrxh3 and the AtTrxh3-mediated physiological effects of ABA-binding to AtTrxh3 remain to be elucidated (Anabuki et al. 2022). It is conceivable that binding of ABA to AtTrxh3 could affect biological processes such as seed germination, as well as plant adaptations to stresses (e.g. heat shock and cold) (Lee et al. 2009; Li et al. 2009) and this would be consistent with a regulatory or tuning role of ABA. Thirdly, ABA also binds directly and specifically to the *A. thaliana* guard cell outward rectifying K^+ channel GORK (At5g37500) and directly promotes K^+ efflux (Ooi et al. 2017) and consequently, stomatal closure. This response might occur at the onset of biotic or abiotic stresses. Incidentally, a stelar K^+ outward rectifying channel (SKOR, At3g02850) also contains a GORK-like ABA-binding site (Ooi et al. 2017) and may also enable direct ABA binding causing K^+ efflux.

What these and other examples suggest is that firstly, direct ABA binding is not likely an ancient relic since it occurs in stomata and the vascular bundle and secondly,

the PYR/PYL/RCAR family and associated PP2C phosphatases are likely not the only mechanism through which specific ABA-dependent responses occur. Moreover, there is also considerable interest in ABA binding in species other than plants and therefore, reasons to extend the hypothesis to animal systems (Lievens et al. 2017). What remains particularly intriguing is the fact that ABA is also present in animals (Le Page-Degivry et al. 1986) and given that animals do not have a PYR/PYL/RCAR signaling system, they may provide us with important clues for the elucidation of alternative ABA-dependent mechanisms. Thus, a systematic approach beginning with sequence analyses of known ABA-binding proteins from both animals and plants would be a rational approach to capturing a more comprehensive ABA-binding protein repertoire (Wong et al. 2018).

How to establish an ABA-binding protein repertoire

Firstly, we propose the use of a computational method to identify candidate ABA-binding proteins. This approach is based on the use of consensus amino acid search terms carefully constructed from binding sites of annotated and/or experimentally validated ABA receptors or ABA-binding proteins. This motif search strategy has in the past been applied to identify the first plant mononucleotide cyclases (Ludidi and Gehring 2003), and more recently also novel plant phosphodiesterases (Kwiatkowski et al. 2021a; b) and gas sensing hemoproteins (Wong et al. 2021a).

Since experimentally tested ABA-binding proteins have been reported, we can probe the ligand-binding centers for conserved residues, extract consensus search terms, and use the constructed term (motif) to query proteomes for candidates. Such a conserved term {D. [7,8]R.[3,4]D.[5,6]Y.[6,7] H (Motif I)} that resembles the latch part of PYR/PYL/PCAR ABA receptors as well as the GORK ABA-binding site (Fig. 1A) was extracted and used to query the *A. thaliana* proteome. It occurs in 30 proteins (see Supplementary Information) and these proteins are enriched in the following gene ontology (GO) terms: "Protein folding", "Heat shock protein binding", and "Unfolded protein binding" (Fig. 1B and Supplementary Information).

As a relatively small, nonpolar, and neutral amino acid, proline does not normally have crucial ligand interaction roles despite being conserved in the motif. A search motif employed previously for the identification for gas sensing hemoproteins has excluded proline from the motif to afford a more comprehensive list of candidates (Wong et al. 2021b). Thus, if the central proline is omitted from the motif (Motif II), we found that this less stringent motif occurs in 182 proteins in the *A. thaliana* proteome (see

Supplementary Information) and noted enrichments in the GO categories "RNA polyadenylation" and "Protein folding" as well as "Nitrogen compound metabolic process" (Fig. 1C). It may imply direct involvement of ABA in these processes. In addition, we also noted ABA-binding sites in *bona fide* ABA downstream components and proteins associated with responses to ABA. This points to an increased complexity in the tuning of ABA-dependent responses (Fig. 2) that awaits experimental testing in vitro and in planta.

Secondly, candidate ABA-binding proteins identified with the ABA search motif will have to be modelled and assessed structurally to establish if a fold that is compatible with ABA binding could be formed. A series of computational approaches that include molecular docking simulations can be performed to evaluate the ABA docking poses, the predicted free energies, and the interactions with key amino acids in the ABA motif. Both visual structural assessments and the statistical values generated by the docking algorithms, lend confidence to the selection of the most promising candidates for in vitro testing. For instance, structural evaluations of representative ABA-binding candidates from *Homo sapiens*: son of sevenless homolog 2, SOS2 (UniProt: Q07890), and exostosin-1, EXT1 (UniProt: Q16394), and from *A. thaliana*: serine/threonine-protein kinase, SRK2D/SnRK2.2 (UniProt: Q39192), and serine/threonine-protein kinase, SRK2E/SnRK2.6 (UniProt: Q940H6), revealed that the predicted ABA-binding region identified by the motif, could form a distinct cavity that can spatially fit the ABA ligand. Subsequent docking simulations on these candidates showed that ABA could conceivably dock at these sites and assume binding poses that allow for interactions with at least two key amino acids in the motif (Fig. 3). We note that while conserved, proline does not seem to be involved in direct interactions with ABA thus justifying the rationale for its exclusion from the motif and the consequent increase in potential candidates. The key amino acids and several others in proximity of the ABA ligand at the binding site can be selected for mutagenesis studies to ascertain the molecular and biological relevance such as the intra- and inter-domain regulations of cellular signaling components. These studies may also elucidate the broader effects on plant development and responses to biotic and abiotic stresses (Zhou et al. 2021).

Thirdly, candidate ABA-binding proteins need to be cloned, heterologously expressed and tested in vitro for binding with both the physiologically active (±)ABA and inactive (−)ABA e.g., by surface plasmon resonance or ELISA. In candidates with multiple domains, the effects of ABA-binding on the neighbouring domains can also be investigated through mutagenesis studies. If a neighbouring domain has e.g., a kinase or adenylate cyclase activity, the
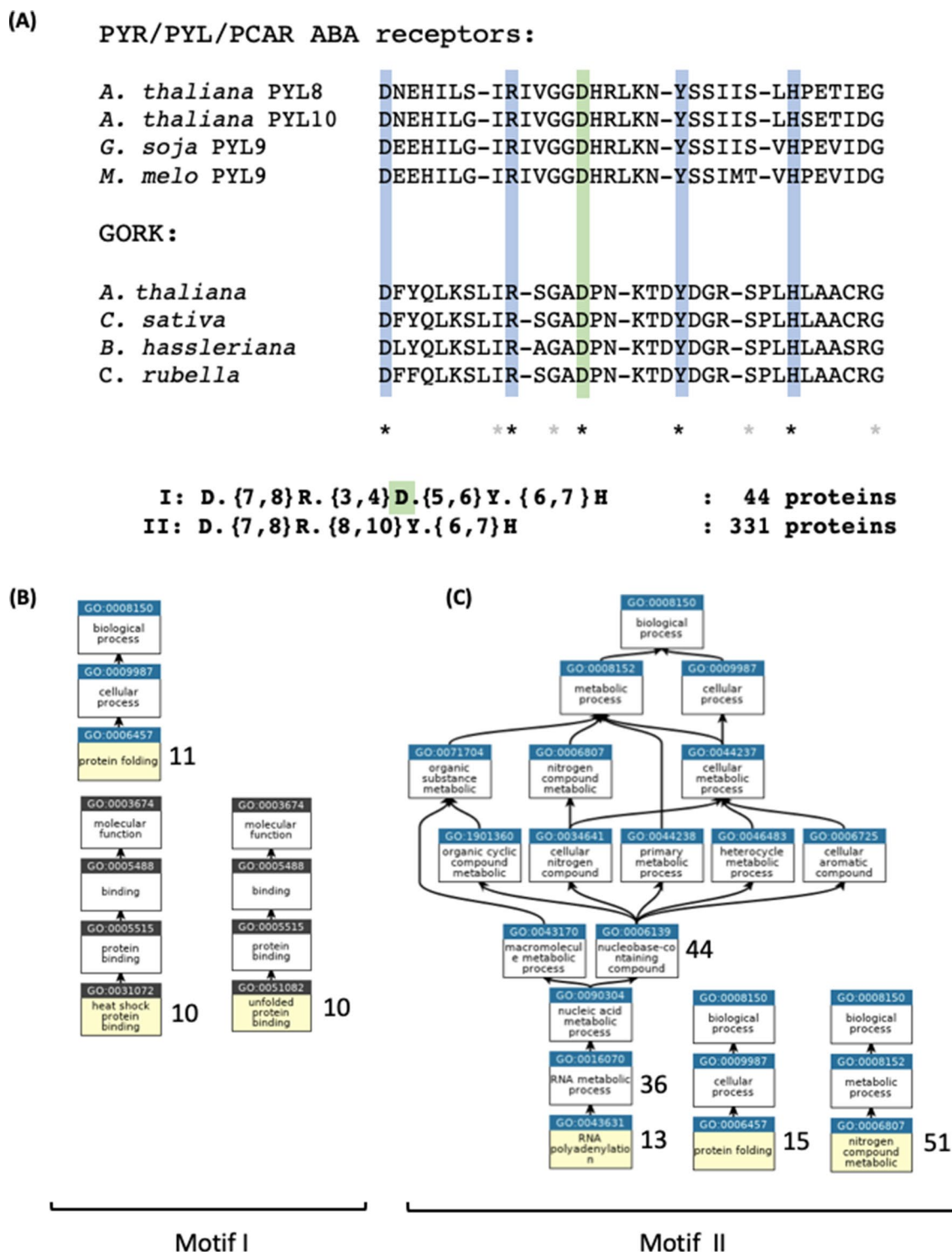
Fig. 1 The ABA-binding motif and GO categories of candidate ABA-binding proteins. Alignment of the PYR/PYL/PCAR latch region of ABA receptors: *A. thaliana* PYL8 (At5g53160, 101–132) and PYL10 (At4g27920, 97–129), *Glycine soja* PYL 9 (XP_028239413.1, 97–129) and *Cucumis melo* (XP_008437914.1, 97–129). ABA-binding domain of GORK channels from *A. thaliana* (At5g37500, 543–575), *Camelia sativa* GORK (XP_010435584.2, 489–521), *Terenaya hassleriana* GORK (XP_010553885.1, 548–580) and *Capsella rubella* GORK (XP_006286320.2, 556–597). The highlighted amino acids (blue) represent residues critical for the interaction with ABA. The proline (**D**, green) is conserved but does not appear to be critical for the interaction with ABA. Asterisks denote conserved amino acids. The two consensus motifs (I and II) are used to query proteomes (**A**). The gene ontology (GO) analysis of the *A. thaliana* genome was performed with agriGO (http://bioinfo.cau.edu.cn/agriGO/) and selected statistically significant enrichments are shown (**B** and **C**). The extended results are shown in the Supplementary Information
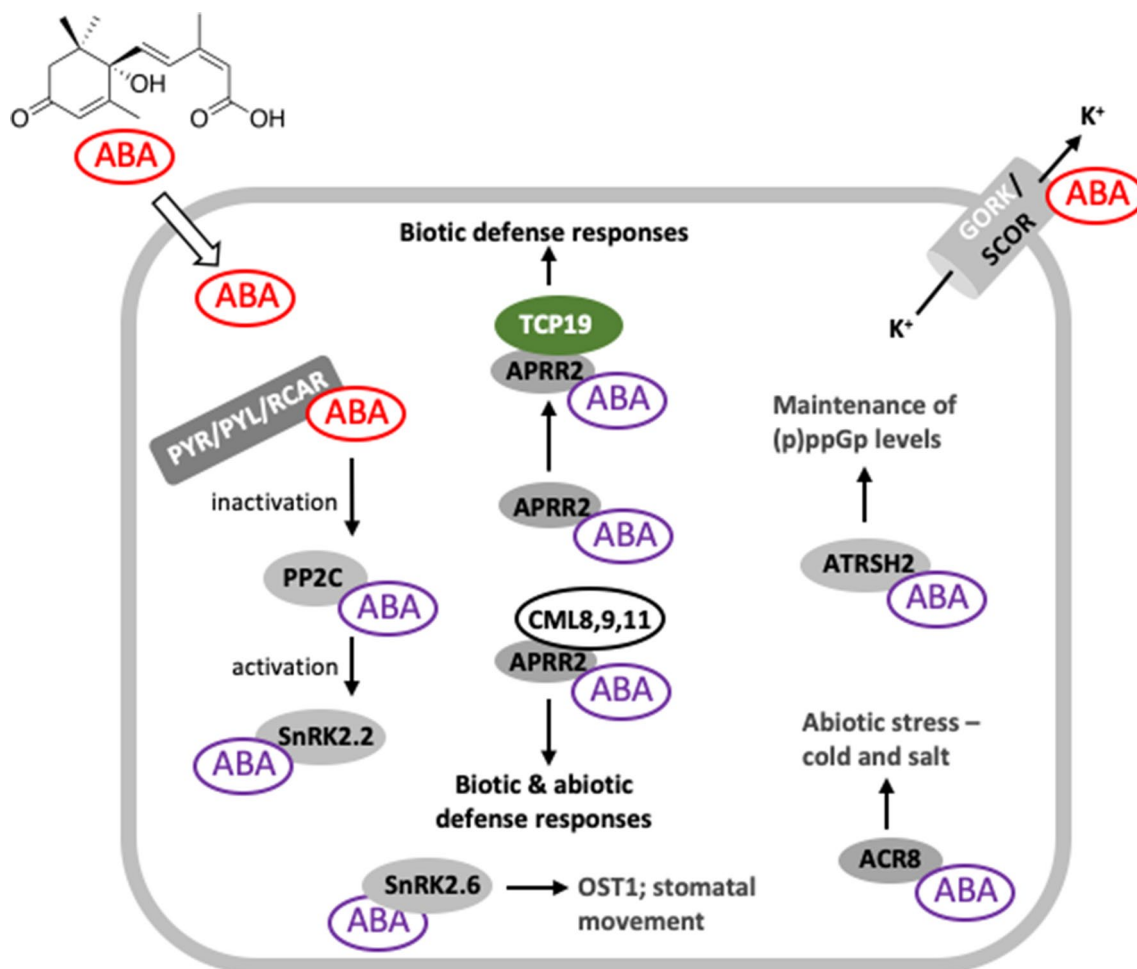
Fig. 2 ABA-binding proteins and selected ABA-binding candidates. The experimentally proven ABA-binding proteins are represented in red and candidate proteins are represented in magenta. The candidates include downstream ABA signaling components PP2C (At3g17090) and SnRK2.2 (At3g50500) as well as SnRK2.6 (At4g33950, Open Stomata 1, OST1). Other ABA-binding candidate proteins include APRR2 (At4g18020), a pseudo response regulator that interacts with calmodulin-like proteins 8, 9 & 11 and the transcription factor TCP19 (green; At5g51910) that is annotated as regulator of defense responses. The binding candidate ACT-domain repeat ACR8 (At1g12420) is implicated in abiotic stress responses and ATRSH2 (At3g14050) is implicated in guanosine 3',5'-bis(pyrophosphate) (ppGpp) homeostasis

effect of ABA-binding on the enzyme activity will have to be tested to unravel specific ABA-dependent biological roles.

Conclusions

If many more direct ABA interactors exist and are shown experimentally to bind and respond to ABA, this will imply that the regulation and tuning of ABA-dependent processes is considerably more complex than currently thought. In plants, candidates that immediately spring to mind are downstream signaling components of the canonical PYR/PYL/RCAR receptors such as SnRK2.2 and SnRK2.6. Establishing direct and specific ABA-binding protein involvement in RNA metabolic processes and RNA polyadenylation would point to a systems level role for ABA in controlling modifications of the transcriptional program. As such, the biological effects of experimentally validated ABA-binding proteins need to be investigated in vivo using transgenic cells or plants expressing proteins harbouring mutations at the ABA-binding sites that results in reduced or abolished ABA binding.

In animals, predicted ABA-binding candidates include proteins with a role in immune responses and tumorigenesis (see Supplementary Information). The establishment of an experimentally confirmed *H. sapiens* ABA-binding protein repertoire will expand and clarify the current role of ABA-dependent processes including immune responses and tumor cell progression. Finding hidden ABA-binding sites will in turn guide further refinement of the ABA
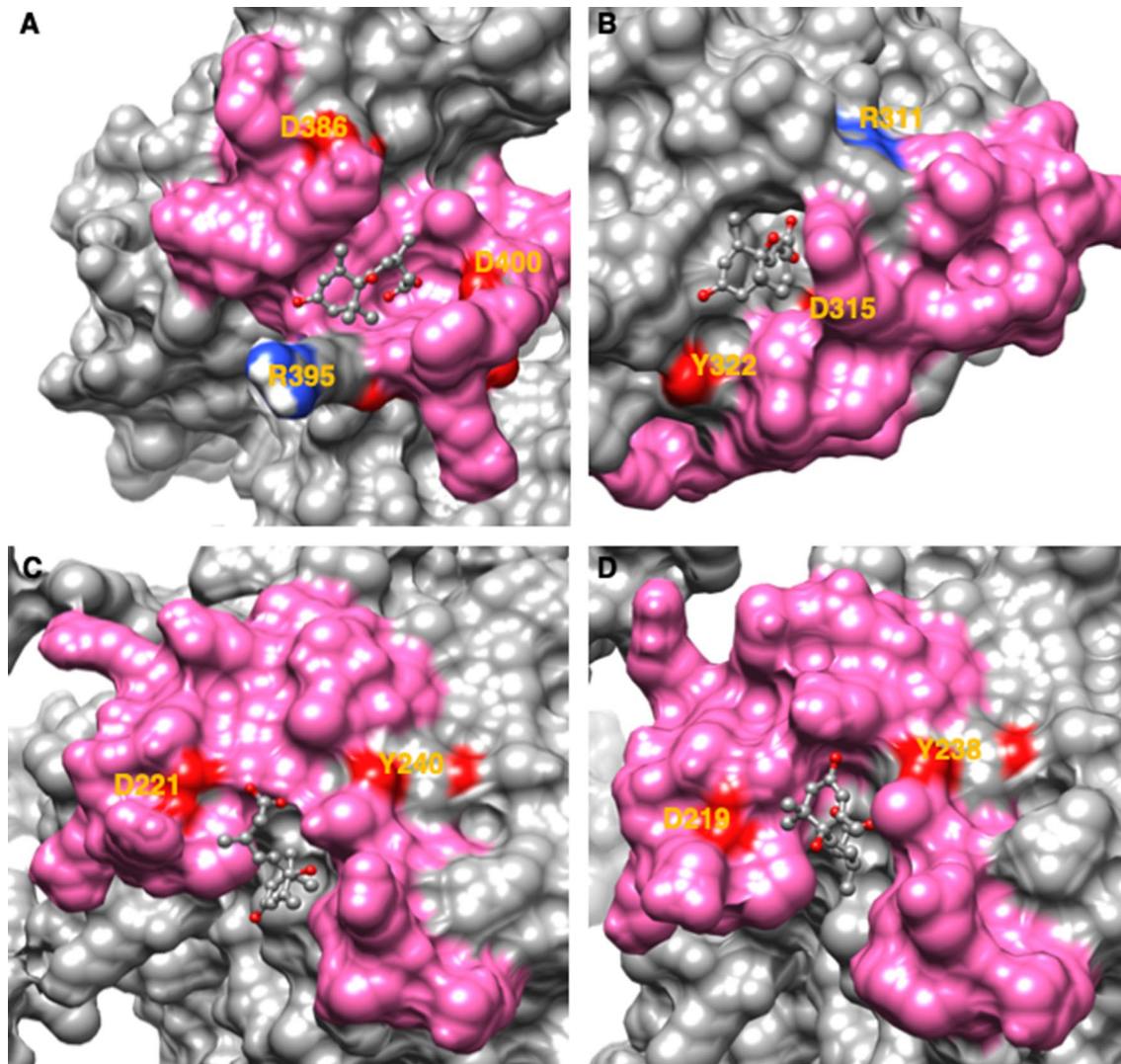
Fig. 3 Structural evaluation of selected candidate ABA-binding proteins from *H. sapiens* and *A. thaliana* retrieved with Motif I or II. Structures of two representative human ABA-binding candidates: son of sevenless homolog 2, SOS2 (UniProt: Q07890) (**A**) and exostosin-1, EXT1 (UniProt: Q16394) (**B**) are shown as surface models. Two representative structures of *A. thaliana* ABA-binding candidates: serine/threonine-protein kinase, SRK2D/SnRK2.2 (UniProt: Q39192, TAIR: At3g50500) (**C**) and serine/threonine-protein kinase, SRK2E/SnRK2.6 (UniProt: Q940H6, TAIR: At4g33950) (**D**) are represented as surface models. The regions corresponding to the ABA-binding amino acid motif are marked in pink, and the conserved amino acids are colored according to their surface charges. In all candidates, the ABA-binding region forms a distinct cavity that can be docked with the ABA ligand. The conserved amino acids in the motif that might interact with ABA are labelled accordingly. For candidates with unresolved crystal structures, the 3D models were obtained from Alpha-Fold (Jumper et al. 2021) prior to ABA docking simulations using AutoDock Vina (ver. 1.1.2) (Trott and Olson 2010). Molecular graphics and analyses were performed with the UCSF Chimera package (Pettersen et al. 2004). Chimera was developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311)

search motif, thereby facilitating the discovery of more ABA interactors. This will likely afford a broader understanding of this hormone and its actions in organisms across species.

Declarations

Conflict of interest The authors declare no competing interests.

References

Ali A, Pardo JM, Yun DJ (2020) Desensitization of ABA-signaling: the swing from activation to degradation. Front Plant Sci 11:1–7. https://doi.org/10.3389/fpls.2020.00379

Anabuki T, Ohashi K, Takasuka TE et al (2022) AtTrxh3, a thioredoxin, is identified as an abscisic acid binding protein in *Arabidopsis thaliana*. Molecules 27:161. https://doi.org/10.3390/molecules27010161

Bassaganya-Riera J, Guri AJ, Lu P et al (2011) Abscisic acid regulates inflammation via ligand-binding domain-independent activation of peroxisome proliferator-activated receptor γ. J Biol Chem 286:2504–2516. https://doi.org/10.1074/jbc.M110.160077

Bauer H, Mayer H, Marchler-Bauer A et al (2000) Characterization of p40/GPR69A as a peripheral membrane protein related to the lantibiotic synthetase component C. Biochem Biophys Res Commun 275:69–74. https://doi.org/10.1006/bbrc.2000.3260

Chen K, Li GJ, Bressan RA et al (2020) Abscisic acid dynamics, signaling, and functions in plants. J Integr Plant Biol 62:25–54. https://doi.org/10.1111/jipb.12899

Chong GL, Foo MH, Lin WD et al (2019) Highly ABA-induced 1 (HAI1)-interacting protein HIN1 and drought acclimation-enhanced splicing efficiency at intron retention sites. Proc Natl Acad Sci USA 116:22376–22385. https://doi.org/10.1073/pnas.1906244116

Cornforth JW, Milborrow BV, Ryback G (1965) Synthesis of (±)—abscisin II. Nature 206:715. https://doi.org/10.1038/206715a0

Cornforth JW, Milborrow BV, Ryback G, Wareing PF (1965) Chemistry and physiology of 'Dormins' in sycamore: identity of sycamore 'Dormin' with abscisin II. Nature 205:1269–1270. https://doi.org/10.1038/2051269b0

Galka MM, Rajagopalan N, Buhrow LM et al (2015) Identification of interactions between abscisic acid and ribulose-1,5-bisphosphate carboxylase/oxygenase. PLoS ONE 10:1–21. https://doi.org/10.1371/journal.pone.0133033

Jumper J, Evans R, Pritzel A et al (2021) Highly accurate protein structure prediction with AlphaFold. Nature 596:583–589. https://doi.org/10.1038/s41586-021-03819-2

Jung Y, Cackowski FC, Yumoto K et al (2021) Abscisic acid regulates dormancy of prostate cancer disseminated tumor cells in the bone marrow. Neoplasia 23:102–111. https://doi.org/10.1016/j.neo.2020.11.009

Kharenko OA, Boyd J, Nelson KM et al (2011) Identification and characterization of interactions between abscisic acid and mitochondrial adenine nucleotide translocators. Biochem J 437:117–123. https://doi.org/10.1042/BJ20101898

Kwiatkowski M, Wong A, Kozakiewicz A et al (2021) A tandem motif-based and structural approach can identify hidden functional phosphodiesterases. Comput Struct Biotechnol J 19:970–975. https://doi.org/10.1016/j.csbj.2021.01.036

Kwiatkowski M, Wong A, Kozakiewicz-Piekarz A et al (2021) In search of monocot phosphodiesterases: identification of a calmodulin stimulated phosphodiesterase from *Brachypodium distachyon*. Int J Mol Sci 22(17):9654. https://doi.org/10.3390/ijms22179654

Le Page-Degivry M-T, Bidard JN, Rouvier E et al (1986) Presence of abscisic acid, a phytohormone, in the mammalian brain. Proc Natl Acad Sci USA 83:1155–1158. https://doi.org/10.1073/pnas.83.4.1155

Lee SY, Park SK, Jung YJ et al (2009) Heat-shock and redox-dependent functional switching of an h-type arabidopsis thioredoxin from a disulfide reductase to a molecular chaperone. Plant Physiol 150:552–561. https://doi.org/10.1104/pp.109.135426

Li YC, Ren JP, Cho MJ et al (2009) The level of expression of thioredoxin is linked to fundamental properties and applications of wheat seeds. Mol Plant 2:430–441. https://doi.org/10.1093/mp/ssp025

Lievens L, Pollier J, Goossens A et al (2017) Abscisic acid as pathogen effector and immune regulator. Front Plant Sci 8:1–15. https://doi.org/10.3389/fpls.2017.00587

Lin Z, Li Y, Wang Y et al (2021) Initiation and amplification of SnRK2 activation in abscisic acid signaling. Nat Commun. https://doi.org/10.1038/s41467-021-22812-x

Lipp J (1990) Nachweis und Herkunft von Abscisinsäure und Prolin in Honig. Apidologie 21:249–259. https://doi.org/10.1051/apido:19900310

Ludidi N, Gehring C (2003) Identification of a novel protein with guanylyl cyclase activity in *Arabidopsis thaliana*. J Biol Chem 278:6490–6494. https://doi.org/10.1074/jbc.M210983200

Mittelheuser CJ, van Steveningk RFM (1969) Stomatal closure and inhibition of transpiration induced by (RS)—abscisic acid. Nature 221:281–282. https://doi.org/10.1038/221281a0

Nakashima K, Yamaguchi-Shinozaki K (2013) ABA signaling in stress-response and seed development. Plant Cell Rep 32:959–970. https://doi.org/10.1007/s00299-013-1418-1

Negri P, Maggi MD, Ramirez L et al (2015) Abscisic acid enhances the immune response in *Apis mellifera* and contributes to the colony fitness. Apidologie 46:542–557. https://doi.org/10.1007/s13592-014-0345-7

Ooi A, Lemtiri-Chlieh F, Wong A, Gehring C (2017) Direct modulation of the guard cell outward-rectifying potassium channel (GORK) by abscisic acid. Mol Plant 10:1469–1472. https://doi.org/10.1016/j.molp.2017.08.010

Pettersen EF, Goddard TD, Huang CC et al (2004) UCSF Chimera—a visualization system for exploratory research and analysis. J Comput Chem 25:1605–1612. https://doi.org/10.1002/jcc.20084

Sturla L, Fresia C, Guida L et al (2009) LANCL2 is necessary for abscisic acid binding and signaling in human granulocytes and in rat insulinoma cells. J Biol Chem 284:28045–28057. https://doi.org/10.1074/jbc.M109.035329

Suzuki Y, Fujimori T, Kanno K et al (2012) Metabolome analysis of photosynthesis and the related primary metabolites in the leaves of transgenic rice plants with increased or decreased Rubisco content. Plant Cell Environ 35:1369–1379. https://doi.org/10.1111/j.1365-3040.2012.02494.x

Takahashi Y, Zhang J, Hsu PK et al (2020) MAP3Kinase-dependent SnRK2-kinase activation is required for abscisic acid signal

transduction and rapid osmotic stress response. Nat Commun 11(1):12. https://doi.org/10.1038/s41467-019-13875-y

Takezawa D, Komatsu K, Sakata Y (2011) ABA in bryophytes: how a universal growth regulator in life became a plant hormone? J Plant Res 124:437–453. https://doi.org/10.1007/s10265-011-0410-5

Trott O, Olson AJ (2010) Autodock vina. J Comput Chem 31:2967–2970. https://doi.org/10.1002/jcc

Vigliarolo T, Guida L, Millo E et al (2015) Abscisic acid transport in human erythrocytes. J Biol Chem 290:13042–13052. https://doi.org/10.1074/jbc.M114.629501

Wang ZY, Gehring C, Zhu J et al (2015) The arabidopsis vacuolar sorting receptor1 is required for osmotic stress-induced abscisic acid biosynthesis. Plant Physiol 167:137–152. https://doi.org/10.1104/pp.114.249268

Wong A, Tian X, Gehring C et al (2018) Discovery of novel functional centers with rationally designed amino acid motifs. Comput Struct Biotechnol J 16:70–76. https://doi.org/10.1016/j.csbj.2018.02.007

Wong A, Hu N, Tian X et al (2021) Nitric oxide sensing revisited. Trends Plant Sci 26:885–897. https://doi.org/10.1016/j.tplants.2021.03.009

Wong A, Tian X, Yang Y et al (2021) Identification of potential nitric oxide-sensing proteins using the H-NOX motif. Mol Plant 14:195–197. https://doi.org/10.1016/j.molp.2020.11.015

Zhou W, Chi W, Shen W et al (2021) Computational identification of functional centers in complex proteins: a step-by-step guide with examples. Front Bioinform 1:652286. https://doi.org/10.3389/fbinf.2021.652286

Zocchi E, Carpaneto A, Cerrano C et al (2001) The temperature-signaling cascade in sponges involves a heat-gated cation channel, abscisic acid, and cyclic ADP-ribose. Proc Natl Acad Sci USA 98:14859–14864. https://doi.org/10.1073/pnas.261448698