



Arabidopsis 14-3-3 proteins: fascinating and less fascinating aspects

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14-3-3 Dimers are well known to interact with diverse target proteins throughout eukaryotes. Most notably, association of 14-3-3s commonly requires phosphorylation of a serine or threonine residue within a specific sequence motif of the client protein. Studies with a focus on individual target proteins have unequivocally demonstrated 14-3-3s to be the crucial factors modifying the client's activity state upon phosphorylation and, thus, finishing the job initiated by a kinase. In this respect, a recent in-depth analysis of the rice transcription factor FLOWERING LOCUS D1 (OsFD1) revealed 14-3-3s to be essential players in floral induction. Such fascinating discoveries, however, can often be ascribed to the random identification of 14-3-3 as an interaction partner of the favorite protein. In contrast, our understanding of 14-3-3 function in higher organisms is frustratingly limited, mainly due to an overwhelming spectrum of putative targets in combination with the existence of a multigene 14-3-3 family. In this review we will discuss our current understanding of the function of plant 14-3-3 proteins, taking into account recent surveys of the *Arabidopsis* 14-3-3 interactome.

Keywords: 14-3-3, interactome

14-3-3 Proteins mediate the effects of certain protein kinases through their ability to bind well-defined phosphoserine or phosphothreonine containing peptide motifs (Muslin et al., 1996; Yaffe et al., 1997; Johnson et al., 2010), the coordination of which finally results in modification of the activity, stability, subcellular localization or interaction capability of the client protein (reviewed in Chevalier et al., 2009; Gökirmak et al., 2010). The fact that 14-3-3s form clamp-like dimers with each monomer capable of binding a phosphopeptide within an amphipathic groove (see **Figure 1**) immediately suggests that 14-3-3s function as an intermolecular bridge linking two different phosphoproteins. However, evidence that 14-3-3s act as adapters is surprisingly limited. More commonly, a 14-3-3 dimer seems to engage with two tandem phosphorylated sites in the same protein (Johnson et al., 2010).

Research in recent years has revealed an impressive list of putative 14-3-3 targets in both animals and plants (reviewed in MacKintosh, 2004; Chevalier et al., 2009; Oecking and Jaspert, 2009; Gökirmak et al., 2010; Denison et al., 2011; Johnson et al., 2011). The emerging picture is that 14-3-3s may function as dynamic coordinators of cellular responses to distinct signaling cues, due to their concerted regulation of cellular signaling, organization, and metabolism. In other words, depending on the environmental and cellular stimuli perceived by a cell, a specific subset of 14-3-3 clients become phosphorylated and – upon 14-3-3 association – brings about an integrated cellular response. Since 14-3-3 association normally relies on phosphorylation, such a scenario can in principle be achieved by 14-3-3 dependent regulation of almost all

the substrates of particular kinases/phosphatases and/or of the kinases/phosphatases themselves.

Historically, plant 14-3-3s were thought to play a prominent role in primary metabolism (reviewed in Huber et al., 2002; Comparot et al., 2003; Denison et al., 2011) and ion homeostasis. Several recent high-throughput proteomic studies, however, have suggested them to also be key regulatory components in signaling cascades, in particular phytohormone mediated processes. Since some of these 14-3-3 interactions have already been reviewed (Chevalier et al., 2009; Oecking and Jaspert, 2009; Gökirmak et al., 2010; Denison et al., 2011) we will focus on recently identified putative interactors in *Arabidopsis* that represent the “historical” and “novel” 14-3-3 targets, namely transporters and proteins involved in hormonal signaling.

DO 14-3-3s FINE-TUNE MEMBRANE TRANSPORT PROCESSES?

While defined K⁺-channels (Sottocornola et al., 2006; Latz et al., 2007; Voelker et al., 2010) and the plasma membrane localized P-type H⁺-ATPase are among the “old players” in the *Arabidopsis* 14-3-3 field (reviewed in Oecking and Jaspert, 2009; Denison et al., 2011), recent studies have suggested a much wider diversity of target proteins involved in membrane transport processes than had previously been realized (Chang et al., 2009; Shin et al., 2011). **Table 1** summarizes these analyses, including data obtained via a yeast two-hybrid screen in our lab (Oecking et al., unpublished, see **Table A1** in Appendix). There are several exciting aspects that should be highlighted. The (putative) 14-3-3 clients include:

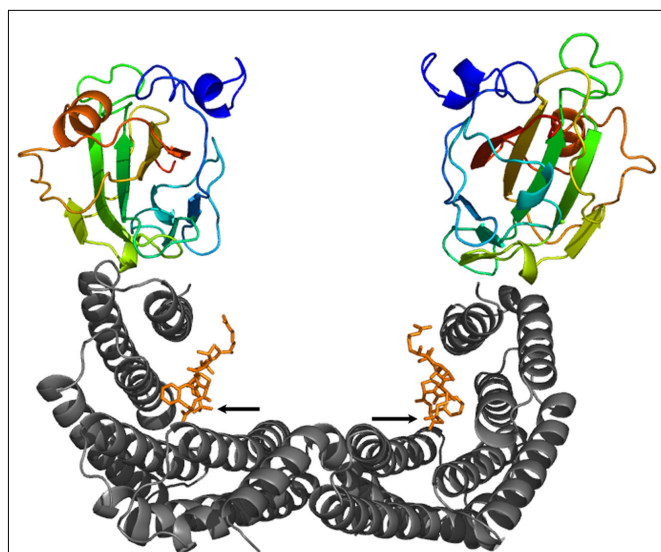


FIGURE 1 | Ribbon plot of a 14-3-3 dimer (gray) in complex with two phosphopeptides derived from the transcription factor OsFD1 (orange, the phosphorylated residue is indicated by an arrow, respectively) and two Hd3a proteins [color coded from the N-terminus (blue) to the C-terminus (red)] (Taoka et al., 2011). While binding of the OsFD1 phosphopeptides occurs in an extended conformation within the amphipathic groove of each 14-3-3 monomer, the “florigen” Hd3a attaches to the “outside” of the groove, respectively.

- (i) All three proton-pumps found in plant cells [i.e., P-type *Arabidopsis* H⁺-ATPase (AHA), V-type H⁺-ATPase (VHA), and the H⁺-pyrophosphatase (H⁺-PPase)]. These primary active transporters establish proton gradients across plasma and endosomal membranes, thus enabling secondary active transport processes and/or endosomal acidification (Gaxiola et al., 2007). This suggests 14-3-3s to be of central importance for the interdependent posttranslational regulation of the cellular complement of proton-pumps. However, with the exception of the P-type H⁺-ATPase which is activated by 14-3-3s (reviewed in Duby and Boutry, 2009), the functional consequences of their interaction with the remaining proton-pumps are not yet known. Furthermore, the major role of the H⁺-PPase AVP1 has recently been shown to rely on removal of inhibitory cytosolic PP_i rather than acidification of the vacuole (Ferjani et al., 2011).
- (ii) Other members of the P-type ATPase family (reviewed in Axelsen and Palmgren, 2001) that transport calcium (ACA or ECA, reviewed in Geisler et al., 2000; Bonza and De Michelis, 2011) or metals (HMA, reviewed in Krämer et al., 2007) and are, thus, essential for cellular ion homeostasis.
- (iii) Almost 20 pumps belonging to the ATP-binding cassette (ABC) family (reviewed in Martinoia et al., 2002), the members of which can carry large organic molecules, including the phytohormone auxin.
- (iv) Several carriers – some of which act as proton symporters [see (i)] – that permit the uptake of nutrients, such as phosphate, sulfate, carbohydrates, and ammonium.

- (v) A multitude of ion channels. Besides the “old players” mediating potassium transport, some poorly selective cation channels (cyclic nucleotide gated channel, CNGC), glutamate receptors which are implicated in cellular Ca²⁺ homeostasis [see (ii)] as well as voltage-dependent anion-selective channels (VDAC) may be subject to 14-3-3 regulation.
- (vi) Finally some aquaporins (reviewed in Maurel et al., 2008) localized either in the plasma membrane (PIP) or the tonoplast (TIP) and known to facilitate the transport of water and/or gases, amongst others.

Taken together, this suggests that 14-3-3 proteins exert an impressively widespread influence on membrane transport processes in plants. Since plants are experts in maintaining optimal metabolic conditions under numerous environmental constraints, 14-3-3s may be the key to a tight and coordinated regulation of transporters that are directly or indirectly involved in ion and nutrient transport. Nevertheless, the number and diversity of these putative 14-3-3 clients are also somewhat intimidating and give rise to numerous questions. Which signals regulate which targets and which kinases are involved? Which mechanisms could elicit a concerted regulation of transporters whose interplay would make sense, such as proton-pumps and secondary active transporters? As the list of 14-3-3 clients is continually growing, the question whether the cellular “14-3-3 pool” is limiting under certain circumstances is becoming more important. However, it should be noted that, in the majority of cases, a mere handful of transporters has been identified. Indeed, the bulk of the above-mentioned clients was discovered by one study (Shin et al., 2011), suggesting that the given developmental stage and growth conditions of the plant material used favor 14-3-3 mediated regulation of membrane transporters.

ARE 14-3-3s GENERAL REGULATORS OF PHYTOHORMONE MEDIATED PROCESSES?

Meanwhile, *Arabidopsis* 14-3-3 proteins have been proved to be important mediators in signaling cascades (reviewed in Chevalier et al., 2009; Oecking and Jaspert, 2009; Gökirmak et al., 2010; Denison et al., 2011). This breakthrough was achieved by several elegant studies demonstrating 14-3-3s to be essential players in brassinosteroid (BR) signaling due to their modification of the subcellular localization of key transcription factors, such as brassinazole resistant1 (BZR1; Gampala et al., 2007; Ryu et al., 2007) and BRI1 EMS suppressor1 (BES1; Ryu et al., 2010). Currently available *Arabidopsis* 14-3-3 interactome data moreover suggest 14-3-3s to participate in processes mediated by almost any phytohormone (Chang et al., 2009; Paul et al., 2009; Shin et al., 2011; Swatek et al., 2011; summarized in **Table 2** which includes data obtained via a yeast two-hybrid screen in our lab; Oecking et al., unpublished, see **Table A1** in Appendix). First of all, not only a multitude of further proteins likewise involved in BR signaling but also polypeptides critical for its biosynthesis have been identified as potential 14-3-3 clients. A comparable scenario emerges with respect to the gaseous hormone ethylene (**Table 2**). At present, we can only speculate as to why multiple 14-3-3 interactions might be required to control a defined cellular response. One conceivable

Table 1 | A summary of prominent membrane transporters recently identified as putative Arabidopsis 14-3-3 interactors.

Category of transporter	Putative 14-3-3 client	Transported molecule	Detection of interaction	Reference
PUMP				
P-type ATPase	AHA1, AHA2	H ⁺	Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	AHA2		Y2H	Arabidopsis Interactome Mapping Consortium (2011)
	AHA2, AHA3, AHA10		AC	Shin et al. (2011)
	AHA6		TAP, Y2H	Chang et al. (2009), Arabidopsis Interactome Mapping Consortium (2011)
V-type ATPase	ACA2, ECA1, ECA2	Ca ²⁺	AC	Shin et al. (2011)
	HMA4	Heavy metals	TAP, Y2H	Chang et al. (2009), Arabidopsis Interactome Mapping Consortium (2011)
	HMA6, HMA7		AC	Shin et al. (2011)
	VHA-A, VHA-B2	H ⁺	AC	Shin et al. (2011)
H ⁺ -PPase	VHA-H		Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	AVP1	H ⁺	Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
ABC transporter	AVP2		AC	Shin et al. (2011)
	20 Isoforms belonging to subgroups A, B, C, or G	Organic molecules	AC	Shin et al. (2011)
CARRIER				
H ⁺ -symporter	SUC6, TMT1, STP14	Carbohydrates	AC	Shin et al. (2011)
	STP4		Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	SULTR1;3, SULTR2;1, SULTR3;1, SULTR4;1	SO ₄ ²⁻	AC	Shin et al. (2011)
	PHT1;1, PHT1;6	PO ₄ ²⁻	AC	Shin et al. (2011)
	PHT3;1		Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	CAX2	Ca ²⁺	AC	Shin et al. (2011)
Facilitator	AMT1;1	NH ₄ ⁺	AC	Shin et al. (2011)
	COPT1	copper	Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	KUP6, KUP7	K ⁺	AC	Shin et al. (2011)
	MGT2	Magnesium	Y2H	Arabidopsis Interactome Mapping Consortium (2011)
	ZIP10	Zinc	AC	Shin et al. (2011)
CHANNEL				
Tandem pore K ⁺ channel	TPK1	K ⁺	GST-PD, SPR	Latz et al. (2007)
	TPK3		AC	Shin et al. (2011)
	TPK1, TPK5		Y2H, BiFC	Voelker et al. (2010)
Shaker K ⁺ channel	KAT1	K ⁺	<i>In vitro</i> overlay	Sottocornola et al. (2006)
	AKT2, AKT5		AC	Shin et al. (2011)
	GORK		TAP, Y2H	Chang et al. (2009), Arabidopsis Interactome Mapping Consortium (2011)
Cyclic nucleotide gated channel (CNGC)	Isoforms 5, 6, 10, 18	Cations	AC	Shin et al. (2011)
	isoform 17		Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
Glutamate receptor	Seven different isoforms	Ca ²⁺ ?	TAP, Y2H, AC	Chang et al. (2009), Arabidopsis Interactome Mapping Consortium (2011), Shin et al. (2011)
Voltage-dependent anion channel	VDAC1, VDAC2, VDAC3	Anions	Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
Aquaporin	PIP1;2, PIP1;5, PIP2;7, TIP1;2, TIP2;1	H ₂ O, gases	Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)

AC, 14-3-3 affinity chromatography of plant extracts; BiFC, bimolecular fluorescence complementation; PD, pull-down assay; SPR, surface plasmon resonance; TAP, tandem affinity purification of 14-3-3 protein complexes; Y2H yeast two-hybrid.

Table 2 | A summary of recently identified putative *Arabidopsis* 14-3-3 interactors involved in phytohormone signaling or biosynthesis.

Hormone pathway	Putative 14-3-3 client	Client's function	Detection of interaction	Reference
BR signaling	BRI1, BRL2, BAK1	Receptor/co-receptor	TAP, Y2H	Chang et al. (2009), <i>Arabidopsis</i> Interactome Mapping Consortium (2011)
	SERK1		Co-IP, Y2H	Karlova et al. (2006), <i>Arabidopsis</i> Interactome Mapping Consortium (2011)
	BSU1, BSL1	Protein phosphatase	TAP, Y2H	Chang et al. (2009), <i>Arabidopsis</i> Interactome Mapping Consortium (2011)
	BZR1	Transcriptional regulator	BiFC, Y2H	Gampala et al. (2007), Ryu et al. (2007), <i>Arabidopsis</i> Interactome Mapping Consortium (2011)
	BES1		Co-IP, Y2H	Ryu et al. (2010), <i>Arabidopsis</i> Interactome Mapping Consortium (2011)
BR biosynthesis	BEE3, BES1, BIM1, BZR1		Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	CYP85A19	BR-6-oxidase	AC	Shin et al. (2011)
Ethylene signaling	CBB1/DWF1	Campesterol formation	Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	ETR1	Receptor	AC	Shin et al. (2011)
	EIN2	Membrane protein	AC	Shin et al. (2011)
	ERF1, ERF9	Transcriptional regulator	AC	Shin et al. (2011)
Ethylene biosynthesis	ERF11		Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	ACS6, ACS7, ACS8	ACC-synthase	TAP, Y2H	Chang et al. (2009), <i>Arabidopsis</i> Interactome Mapping Consortium (2011)
	ACS6, ACS10		AC	Shin et al. (2011)
	ACO2, ACO4	ACC-oxidase	Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	EOL1, EOL2	ETO1-like: direct ACS for degradation	TAP, Y2H	Chang et al. (2009), <i>Arabidopsis</i> Interactome Mapping Consortium (2011)
ABA signaling	EOL2		AC	Shin et al. (2011)
	ABF1, ABF2, ABF3, ABF4, ABI5	Transcriptional regulator	Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
	AREB3		Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix) <i>Arabidopsis</i> Interactome Mapping Consortium (2011)
GA signaling	RGA, RGL2	Transcriptional regulator	AC	Shin et al. (2011)
CK signaling	ARR12	Transcriptional regulator	AC	Shin et al. (2011)
	ARR2		Y2H	<i>Arabidopsis</i> Interactome Mapping Consortium (2011)
	CRF6		Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
CK homeostasis	CKX3	CK oxidase	AC	Shin et al. (2011)
Auxin signaling	ARF6, ARF15, ARF18	Transcriptional regulator	AC	Shin et al. (2011)
	IAA14, IAA17, IAA18, IAA19	Aux/IAA transcriptional regulator	AC	Shin et al. (2011)
	Auxin homeostasis	NIT1, NIT2	Nitrilase	AC
NIT1			Y2H	Oecking et al. (unpublished) (see Table A1 in Appendix)
NIT3			AC	Shin et al. (2011)
GH3.3, GH3.5		IAA amido synthetase	AC	Shin et al. (2011)
GH3.9			AC	Swatek et al. (2011)
	IAR4	IAA-conjugate resistant	AC	Shin et al. (2011)

AC, 14-3-3 affinity chromatography of plant extracts; BiFC, bimolecular fluorescence complementation; Co-IP, co-immunoprecipitation; TAP, tandem affinity purification of 14-3-3 protein complexes; Y2H, yeast two-hybrid.

scenario is that 14-3-3 function as molecular gauges, thereby forcing the cell to react to a given phytohormone according to the signal strength. Taking into account that clients involved in several cellular processes might compete for binding to 14-3-3s, numerous targets within one defined pathway are assumed to channel the overall cellular response.

Remarkably, the modification of transcriptional regulators involved in hormonal signaling seems to be another hotspot in *Arabidopsis* 14-3-3 biology. In this regard, several prominent proteins, which act as either repressors or activators of gene expression in BR (BZR1, BES1, BIM1), ethylene [ethylene responsive factor (ERF)], auxin [auxin/indole-3-acetic acid proteins (Aux/IAA)],

auxin response transcription factor (ARF)], gibberellin (GA) [repressor of GA1-3 (RGA), RGA-like (RGL)], abscisic acid (ABA) [ABA response element binding factor (ABF)], and cytokinin (CK) [*Arabidopsis* response regulator (ARR), cytokinin response factor (CRF)] responses, are among the putative 14-3-3 clients in *Arabidopsis* (Table 2). In contrast to BZR1/BES1, which are retained in the cytosol upon 14-3-3 association (Gampala et al., 2007; Ryu et al., 2007, 2010), the members of the ABF subfamily of BASIC REGION/LEUCINE ZIPPER (bZIP) transcription factors constitutively localize to the nucleus, as shown for ABA INSENSITIVE 5 (ABI5; Lopez-Molina et al., 2002), ABF3 (Sirichandra et al., 2010) and the rice homolog OREB1 (Hong et al., 2011).

In this respect, 14-3-3s have been proposed to be critical for the stability of the ABF3 protein, the proteasomal turnover rate of which is high in the absence of ABA and 14-3-3s (Sirichandra et al., 2010). Even though 14-3-3 dependent modification of transcriptional regulators mediating ethylene, auxin, GA, and CK responses has yet to be verified, 14-3-3s might in summary be far more important for assuring the developmental plasticity of plants than has yet been assumed. The fact that more and more proteins are identified as putative targets moreover implies that 14-3-3 interactions are highly dynamic which would enable the cellular 14-3-3 “pool” to immediately and precisely react to altered signaling cues.

14-3-3s ARE CRITICAL FOR FLORAL TRANSITION IN RICE

While global studies are essential to get an impression of the extent of the plant 14-3-3 interactome, the necessity to address the biological significance of individual interactions is more pressing than ever. In this respect, a recent study elegantly combining biochemistry, cell biology, and genetics has impressively proved 14-3-3s to be essential components of the florigen activation complex (FCA) that promotes flowering in the short day plant rice (Taoka et al., 2011). The term “florigen” was created in 1936 and refers to a molecule that is generated in leaves under inductive photoperiodic conditions and subsequently transported to the shoot apex (Chailakhyan, 1936). Evidence could only recently be provided that the protein flowering locus T (FT) represents such a long-distance signal in the facultative long day plant *Arabidopsis* (Mathieu et al., 2007). In the shoot apex, a complex of FT and the bZIP transcription factor flowering locus D (FD) initiates floral development through transcriptional activation of floral identity genes (Abe et al., 2005; Wigge et al., 2005).

Using the rice FT homolog Hd3a as a bait in a yeast two-hybrid screen, (Taoka et al., 2011) identified rice 14-3-3 isoforms as well as OsFD1, a rice homolog of the *Arabidopsis* FD, as putative binding partners. The initially astonishing observation that Hd3a is unable to interact directly with OsFD1 gave rise to subsequent in-depth analyses demonstrating 14-3-3s to mediate this interaction. Remarkably, the binding sites in 14-3-3 for Hd3a and OsFD1 are separated, indicating that the two partners bind in different manner. OsFD1 represents the typical 14-3-3 target, in that its coordination within the typical groove of a 14-3-3 monomer depends on phosphorylation. However, in contrast with most physiological targets, Hd3a does not have to be phosphorylated in order to associate with 14-3-3s. The obtained crystal structure

of the Hd3a:14-3-3 complex revealed a fascinating difference as compared to the canonical 14-3-3 interactions: an unphosphorylated Hd3a monomer binds “on top” of each 14-3-3 monomer, thus extending the W-shaped structure of the 14-3-3 dimer (Taoka et al., 2011; Figure 1). Crystal soaking in the presence of a phosphorylated OsFD1 peptide representing the 14-3-3 binding site finally allowed the modeling of the FCA holocomplex composed of 14-3-3, OsFD1 and Hd3a. Notably, even though 14-3-3s mediate the Hd3a:OsFD1 interaction, the two proteins do not come in contact with each other, suggesting 14-3-3s to function as a platform enabling spatial proximity but not direct linkage (Taoka et al., 2011). On the basis of cell biological data Taoka et al. (2011) proposed a model according to which 14-3-3s act as cytoplasmic receptors for the “florigen” Hd3a in the shoot apex. Once this protein couple enters the nucleus, a ternary complex including phosphorylated OsFD1 is built, which in turn is retained in the nucleus and activates transcription of genes crucial for floral induction. Finally, several experiments performed with mutant versions of either Hd3a or OsFD1 that had lost their ability to interact with 14-3-3s have impressively proved the *in vivo* significance of 14-3-3 association for flowering in rice.

However, based upon the current structural knowledge nearly each 14-3-3 client should be able to associate with the Hd3a:14-3-3 complex. Thus, one crucial question is still unanswered: what determines specificity of the Hd3a:14-3-3 complex to interact exclusively with OsFD1?

MINOR KNOWLEDGE ABOUT THE BIOLOGICAL ROLE OF DISTINCT 14-3-3 ISOFORMS

The above-mentioned studies, demonstrating that 14-3-3s are of the utmost importance for BR signaling and timing of floral transition, were focused on particular 14-3-3 client proteins and have generated substantial insight into the function of plant 14-3-3s. Considering the enormous quantity and functional complexity of the recently identified putative 14-3-3 targets, the subset of which may vary considerably as a function of the developmental and physiological stage, the question as to whether *Arabidopsis* 14-3-3 mutants can broaden our understanding of 14-3-3 function becomes more and more critical. *Arabidopsis* expresses thirteen 14-3-3 isoforms that can be divided into two major phylogenetic groups, the epsilon and the non-epsilon group, the latter consisting of three organizational subgroups. Since the epsilon group is considered to harbor living fossil isoforms which may fulfill fundamental eukaryotic functions, non-epsilon members may be responsible for organism-specific regulatory aspects.

The current knowledge about plants characterized by reduced or absent expression of specific 14-3-3 isoforms is limited (see Oecking and Jaspert, 2009; Denison et al., 2011). We identified T-DNA induced *loss-of-function* alleles of several individual non-epsilon 14-3-3 isoforms, which collectively do not show a statistically significant phenotype under normal growth conditions. The same applies to the simultaneous *loss-of-function* of two 14-3-3 isoforms (kappa/lambda) constituting a phylogenetic non-epsilon subgroup, suggesting functional redundancy among members of the non-epsilon group at least with respect to fundamental functions (data not shown). Beyond that, although lambda and kappa were the most frequently 14-3-3 isoforms identified

in a yeast two-hybrid screen to interact with BZR1, the corresponding double knockout mutants did not display altered BR responses (Gampala et al., 2007). Taken together, redundancy of all non-epsilon isoforms independent of their belonging to different phylogenetic subgroups is likely. Thus, higher order *loss-of-function* mutants seem to be required to produce a phenotype, which in turn is expected to be pleiotropic and is thus difficult to interpret. Nevertheless, focusing on well-defined developmental stages could provide hints for the dominant and major 14-3-3 targets under the given circumstances.

What about epsilon group members? While many of the recent proteome wide approaches in *Arabidopsis* have focused on the

identification of putative non-epsilon 14-3-3 interactors, only one study compared two phylogenetically distinct isoforms (Swatek et al., 2011). The results suggest not only isoform specificity of several target proteins but also preference for 14-3-3 dimer formation between phylogenetically similar 14-3-3 isoforms. Hence, functional specialization may exist, at least between members of the non-epsilon and epsilon group.

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APPENDIX

Table A1 | Results of a yeast two-hybrid screen using a cDNA library obtained from 3 days old etiolated *Arabidopsis* seedlings (Kim et al., 1997) and different *Arabidopsis* 14-3-3 isoforms as a bait.

Locus	Gene name
MEMBRANE TRANSPORTER	
AT1G15690	H ⁺ -translocating inorganic pyrophosphatase (H ⁺ -PPase) located in the vacuolar membrane (AVP1)
AT2G18960	Plasma membrane H ⁺ -ATPase1 (AHA1)
AT2G45960	Plasma membrane intrinsic protein (PIP1;2)
AT3G01280	Voltage-dependent anion channel 1 (VDAC1)
AT3G16240	Tonoplast intrinsic protein (TIP2;1)
AT3G19930	Sucrose hydrogen symporter, sugar transporter 4 (STP4)
AT3G26520	Tonoplast intrinsic protein (TIP1;2)
AT3G42050	Vacuolar ATPsynthase subunit H family protein (VHA-H)
AT4G30360	Cyclic nucleotide gated channel 17 (CNGC17)
AT4G30190	Plasma membrane H ⁺ -ATPase2 (AHA2)
AT4G35100	Plasma membrane intrinsic protein (PIP2;7)
AT5G14040	Phosphate transporter (PHT3;1)
AT5G15090	Voltage-dependent anion channel (VDAC3)
AT5G59030	Copper transporter 1 (COPT1)
AT5G67500	Voltage-dependent anion channel 2 (VDAC2)
HORMONE SIGNALING/BIOSYNTHESIS	
AT1G05010	ACC-oxidase 4 (ACO4)/ethylene forming enzyme (EFE)
AT1G19350	BR11-EMS-suppressor 1 (BES1)/brassinazole-resistant 2 (BZR2)
AT1G28370	Ethylene response factor 11 (ERF11)
AT1G45249	Abscisic acid responsive elements-binding factor 2 (ABF2)
AT1G49720	Abscisic acid responsive elements-binding factor 1 (ABF1)
AT1G62380	ACC-oxidase 2 (ACO2)
AT1G73830	BR enhanced expression 3 (BEE3)
AT1G75080	Brassinazole-resistant 1 (BZR1)
AT2G36270	Abscisic acid insensitive 5 (ABI5)
AT3G19290	Abscisic acid responsive elements-binding factor 4 (ABF4)
AT3G19820	Cabbage 1 (CBB1)/dwarf 1 (DWF1)
AT3G44310	Nitrilase 1 (NIT1)
AT3G56850	Abscisic acid responsive element binding protein 3 (AREB3)
AT3G61630	Cytokinin response factor 6 (CRF6)
AT4G34000	Abscisic acid responsive elements-binding factor 3 (ABF3)
AT5G08130	BES1-interacting Myc-like protein 1 (BIM1)

Shown are putative 14-3-3 interactors involved in either membrane transport processes or phytohormone signaling/biosynthesis.