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The Arabidopsis 14-3-3 Multigene Family[1]

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The 14-3-3 proteins are ubiquitous eukaryotic proteins and are encoded by a gene family in many species. We examined the 14-3-3 gene family in *Arabidopsis thaliana* and found that it contains 10 members. Four new cDNAs, GF14ϵ, GF14κ, GF14μ, and GF14ν, and two new genomic clones of GF14ϕ and GF14υ were isolated and characterized. Together with the six previously described 14-3-3 isoforms in Arabidopsis, they constitute a complete family of 10 distinct 14-3-3 proteins of 248 to 268 amino acids. Phylogenetic analysis revealed the presence of two ancient, distinct 14-3-3 gene classes in Arabidopsis and other plants. The ϵ forms diverged early from the other plant isoforms, and plant 14-3-3 genes displayed a different evolutionary course from that of mammals.

The 14-3-3 proteins exist as protein families that contain highly conserved, but individually distinct, isoforms in many species (Ferl et al., 1994; Ferl, 1996). They were originally isolated as soluble, cytosolic, and acidic proteins from bovine brain on the basis of their electrophoretic mobilities (Moore and Perez, 1967). Further studies showed that, in addition to their abundance in mammalian brain, 14-3-3 proteins are also present in all eukaryotic organisms examined to date (Aitken, 1995; Ferl, 1996).

An assortment of activities and functions has been attributed to 14-3-3 proteins, most of which are involved in cellular signaling pathways. In mammals there are seven distinct 14-3-3 isoforms that were initially described as phosphorylation-dependent activators of Tyr and Trp hydroxylase during the biosynthesis of serotonin and dopamine (Yamauchi et al., 1981; Ichimura et al., 1987). Later, 14-3-3 proteins were found to regulate protein kinase C activity (Toker et al., 1992; Tanji et al., 1994), to activate calcium-dependent exocytosis in permeabilized adrenal chromaffin cells (Morgan and Burgoyne, 1992), and to associate with and activate Raf-1 kinase, a key member in the mitogen-activated protein kinase cascade (Fantl et al., 1994; Freed et al., 1994; Fu et al., 1994; Irie et al., 1994).

More recently, mammalian 14-3-3 proteins were shown to bind to and be phosphorylated by Bcr kinase (Reuther et al., 1994), to associate with polyoma virus middle T antigen (which in turn associates with other proteins involved in the regulation of cell proliferation; Pallas et al., 1994), and to stimulate mitochondrial import in rats (Alam et al., 1994). The 14-3-3 proteins function as part of the cell cycle-controlled DNA-damage checkpoint in the yeast *Schizosaccharomyces pombe* (Ford et al., 1994), and they form a complex with cdc25 phosphatase (Conklin et al., 1995).

The 14-3-3 proteins were found to associate with the G-box DNA/protein complex in *Arabidopsis thaliana* and maize (*Zea mays*) (de Vetten et al., 1992; Lu et al., 1992), to function as a receptor for the phytotoxin fusicoccin in certain plants (Korthout and de Boer, 1994; Oecking et al., 1994), and to bind and regulate nitrate reductase (Bachmann et al., 1996a, 1996b; Moorhead et al., 1996). Arabidopsis 14-3-3 proteins were also shown to interchangeably carry out certain cellular functions ascribed to mammalian and yeast 14-3-3 proteins (Lu et al., 1994; van Heusden et al., 1995).

At present, the reason such homologous isoforms are responsible for so many diverse functions remains unknown. One leading model is that different isoforms, although they may have many conserved properties, specialize in individual functions on the basis of their isoform diversity, distinct cell-specific and/or developmentally regulated expression, and differential responses to environmental stimuli. In support of this model, some Arabidopsis 14-3-3 proteins demonstrate differential ability to regulate nitrate reductase (Bachmann et al., 1996a).

Six 14-3-3 isoforms have been identified in Arabidopsis in previous studies. Five isoforms were isolated during a screening of an expression library using a monoclonal antibody that recognized a component(s) in a G-box DNA/protein complex (Lu et al., 1992, 1994). These isoforms were identified as 14-3-3 proteins based on their amino acid sequence homology with known 14-3-3 proteins, and were designated as GF14ψ, GF14χ, GF14ϕ, GF14ω, and GF14υ (G-box Factor 14-3-3 homologs). Another isoform, RCI2 (for *R*are *C*old-*I*nducible cDNAs), was identified in a screening of cold-acclimated, etiolated seedlings of Arabidopsis with a subtracted probe (Jarillo et al., 1994). The genomic clones of three GF14 genes were also identified: GRF1-GF14χ, GRF2-GF14ω, and GRF3-GF14ψ (for *G*eneral *R*egulatory

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Abbreviations: dbEST, database of expressed sequence tags; GF14, G-box factor 14-3-3 homolog; GRF, general regulatory factor.

Factor, with numbers designating the order in which the genomic clones were identified) (Rooney and Ferl, 1995; Daugherty et al., 1996).

To better understand the functions of 14-3-3 proteins, it is important to fully characterize certain benchmark organisms with respect to all 14-3-3 proteins present. We isolated and characterized four novel 14-3-3 cDNAs in Arabidopsis: GF14ϵ, GF14κ, GF14μ, and GF14ν. Together with the six known 14-3-3 isoforms, they represent the largest complete 14-3-3 protein family in all organisms characterized. The isolation and characterization of two new Arabidopsis GF14 genes is also reported here. Sequence analyses of all 10 Arabidopsis GF14 proteins and other 14-3-3 proteins revealed the presence of two divergent classes of Arabidopsis 14-3-3 genes that may encode specialized functions.

MATERIALS AND METHODS

Isolation of New 14-3-3 Isoforms

Yeast Two-Hybrid Screening

The coding region of GF14χ was amplified by PCR and subcloned into a *Bam*HI site of the two-hybrid shuttle/expression vector pGBT9, as previously described (Wu et al., 1997). The coding region was in-frame as determined by sequencing. The construct was cotransformed into HF7C yeast cells with pGAD10 constructs containing a yeast (*Saccharomyces cerevisiae*) two-hybrid cDNA library of *Arabidopsis thaliana* to identify potential proteins that could interact with GF14χ protein, according to the protocol provided by the manufacturer (Clontech, Palo Alto, CA). The transformants were plated on selection media lacking His, Leu, and Trp. A total of 2×10^6 colonies was screened. Positive colonies that could produce His were isolated and their DNA was subsequently purified and sequenced.

Searching of dbEST

Full-length cDNA sequences of Arabidopsis GF14 proteins were used as probes during a BLAST search of the dbEST of GenBank using the search engine HyperBLAST version 1.10 (National Center for Biotechnology Information, Bethesda, MD). BLAST search parameters included an expected number of matches of 25 and a default scoring matrix. Arabidopsis EST entries with homology to the probes were retrieved and their sequences were compared with the probes to determine their identities. EST entries that were not identical to any of the already characterized Arabidopsis GF14 proteins were analyzed to determine unique sequences. Those representing unique 14-3-3 sequences were ordered from the DNA Stock Center of the Arabidopsis Biological Resource Center (The Ohio State University, Columbus) and DNA was subsequently purified and sequenced.

Isolation of Genomic Clones of 14-3-3

Isolation of genomic clones of Arabidopsis 14-3-3 genes was carried out as previously described (Daugherty et al., 1996). An Arabidopsis genomic library (Clontech) was plated on *Escherichia coli* K803 and screened by hybridization at high stringency with ^{32}P-labeled GF14 cDNAs. The hybridization was performed at 65°C for 18 h in 0.5 M sodium phosphate, pH 7.2, 1 mM EDTA, 7% SDS, and 1% BSA. The filters were washed for 1 h with three changes of 40 mM sodium phosphate, pH 7.2, 24 mM NaCl, 0.1% SDS, and 1 mM EDTA at 65°C, and exposed to XAR-5 film (Kodak) with an intensifying screen. Hybridizing recombinant phages were rescreened until a pure population of plaque was obtained. Phage particles were isolated and DNA was subsequently purified.

Subcloning and Sequencing

Relevant DNA fragments were cloned into pGEM-7Zf(+) vectors and subjected to double-stranded nested deletions (Pharmacia). The nucleotide sequences of both strands of the plasmid DNA were determined by automated dideoxy chain termination (model 373, Applied Biosystems).

Computational Analyses of 14-3-3 Sequences

Nucleotide and amino acid sequences were managed and alignments were performed using GeneWorks (IntelliGenetics, Mountain View, CA). Amino acid identity of the 14-3-3 proteins and coding nucleotide identity of the 14-3-3 coding sequences were also analyzed with GeneWorks. The neighbor-joining and protein parsimony tree-building programs were provided within version 3.5 of the PHYLIP program package (Felsenstein, 1989).

Low-Stringency Southern Analysis

Genomic DNA was isolated from Arabidopsis suspension cells, as described previously (Ferl and Nick, 1987), and then digested with the indicated restriction enzymes, subjected to 0.8% agarose gel electrophoresis, and transferred to Hybond-N^+ nucleic acid transfer membrane (Amersham). The hybridization and washing were performed in a similar manner as described above, except they were carried out at 45°C.

RESULTS

Isolation and Characterization of Four Novel GF14 Isoforms

During a yeast two-hybrid screening of an Arabidopsis cDNA library using GF14χ as bait, seven positive clones were identified. Sequencing of those clones revealed three unique 14-3-3 cDNA sequences. Two of the sequences represented novel 14-3-3 proteins. One of the two novel sequences was designated as GF14κ. Another was designated as GF14ϵ, based on the results from phylogenetic analysis (see below). One of three sequences was identical to RCI2 (Jarillo et al., 1994). To keep the consistency of nomenclature of Arabidopsis 14-3-3s in our study, RCI2 was designated GF14λ.

To further identify and characterize all members of the Arabidopsis GF14 protein family, a BLAST search of Arabidopsis dbEST was carried out using eight known Arabidopsis GF14 cDNA sequences as probes (Table I), on the theory that all characterized 14-3-3 isoforms were highly homologous; 103 EST entries with homology to the probes were retrieved out of a total of 21,056 Arabidopsis EST entries from GenBank (as of October 1995). Of the 103 EST entries, only 17 were not identical to any of the probes at the DNA sequence level. Through sequence comparison, the 17 EST entries could be divided into two groups with unique sequences (data not shown). Eight EST clones from these two groups were ordered and sequenced. Sequencing results showed that the two groups represented two new 14-3-3 isoforms, designated GF14μ and GF14ν.

Each of the four new GF14 cDNAs contains one large open reading frame comprising 248 to 265 amino acid residues. The four novel Arabidopsis GF14 proteins contain 5′ untranslated leaders of 48 to 68 bp and 3′ untranslated regions of 169 to 281 bp, followed by short poly(A)$^+$ tails. There is at least one stop codon in the open reading frame just upstream of the first ATG on the 5′ untranslated leaders of GF14μ and GF14ν (data not shown), which may explain why these two isoforms could not be identified in an expression library.

The Arabidopsis GF14s encode proteins with a calculated M_r of 27,973 to 30,179. The estimated pI of polypeptides range from 4.4 to 4.7, similar to those reported for other 14-3-3 proteins (Nielsen, 1991; Brandt et al., 1992; Zupan et al., 1992). The alignment of the deduced amino acid sequences of Arabidopsis GF14 proteins is shown in Figure 1. The amino acid sequences are highly conserved except in the C-terminal regions. The addition of amino acid sequences of new Arabidopsis GF14 proteins in the alignment does not change the pattern of conserved blocks of amino acids described previously (Ferl et al., 1994). These results are also supported by the observations reported in Table II. As shown in Table II, the percentage of amino acid and coding nucleotide identity of Arabidopsis GF14 proteins ranged from 60 to 92% and 60 to 86%, respectively.

GF14 Gene Structure and Organization

To characterize the organization and structure of the GF14 genes, an Arabidopsis genomic library in λEMBL3 was screened at high stringency with full-length cDNAs of GF14ϕ and GF14ν. Hybridizing phages were isolated and purified. DNA was subsequently isolated and sequenced. The genomic clones were designated as GRF4-GF14ϕ and GRF5-GF14ν, as proposed previously (Rooney and Ferl, 1995; Ferl, 1996).

The genomic structures of the two new Arabidopsis GF14 genes and the three that were previously identified (Rooney and Ferl, 1995) are shown in Figure 2. The genomic sequences of GRF1-GF14χ, GFR2-GF14ω, and GRF4-GF14ϕ reveal three introns in conserved positions (Fig. 2), whereas the genomic sequences of GRF2-GF14ψ and GRF5-GF14ν each display an extra intron in the 5′ untranslated leader of their cDNA sequences in addition to the three introns in the same conserved positions as those of GF14ω, GF14χ, and GF14ϕ (Fig. 2). The positions of introns within the protein-coding sequences are indicated in Figure 1. It is noteworthy that the three conserved intron positions are also present in the two GF14 genomic sequences of maize (*Zea mays*), a monocot (de Vetten and Ferl, 1994). However, unlike those in maize GF14 genes, the introns of the Arabidopsis GF14 genes are not conserved either in length or nucleotide sequences (Fig. 2). In mammals one available genomic sequence of human 14-3-3 η displays a genomic structure very divergent from those in plants. It contains only one intron of approximately 8 kb that is located at the 5′ end of the cDNA sequence (Muratake et al., 1996).

Southern Analyses of the Arabidopsis 14-3-3 Multigene Family

To approach the size and divergence of the 14-3-3 multigene family in Arabidopsis, Southern analysis was performed under low-stringency conditions using probes made from two of the most distantly related genes, GF14ϕ and GF14ϵ. The Arabidopsis genomic DNA restricted with

Table I. *Results of dbEST BLAST search using Arabidopsis 14-3-3 sequences as probes*

14-3-3 Homologs	Match EST Accession Nos.
GF14ψ	R30193 T45017 T88609 H36612 Z33928 H37251 Z25944 T45464 T42452 H36560 R65375 T44005 T21021 T41947
GF14χ	T88244 T42492 Z32630 Z47716 T41737 T21461 Z33784 Z18505 Z29149
GF14ϕ	R29957 T43618 T75753 Z26404 Z30732 T42621 Z30731
GF14ω	T12954 F13837 F13827 T13740 T04075 T03971 T88062 H36108 R29865
GF14ν	H37085 T88418
GF14ε	T20402 Z46557 T88323 T41936 T88028 T44175 T43075 H37302 Z46556
GF14κ	T20470 Z29937 Z34207 T04551 T76574 H37447 T21810 T42214
GF14λ	R64954 R30482 T76099 R36693 F14040 R30460 H36037 T13704 T41754 T41719 R30260 H36731 T42343 R29822 T13629 H36009 F13951 R30565 R64976 T76728 T04438 T75872 T45842 T13899 T41697 T88399 H36742 T88482
GF14μ[a]	T41753 Z25954 T88030 T46314 T21293 R30201 R30326 T76174 T22974 H36714 T76858 H37297 R65479 R65320 Z26527 T22570
GF14ν[a]	T42328

[a] New 14-3-3 homologs identified through dbEST BLAST search.

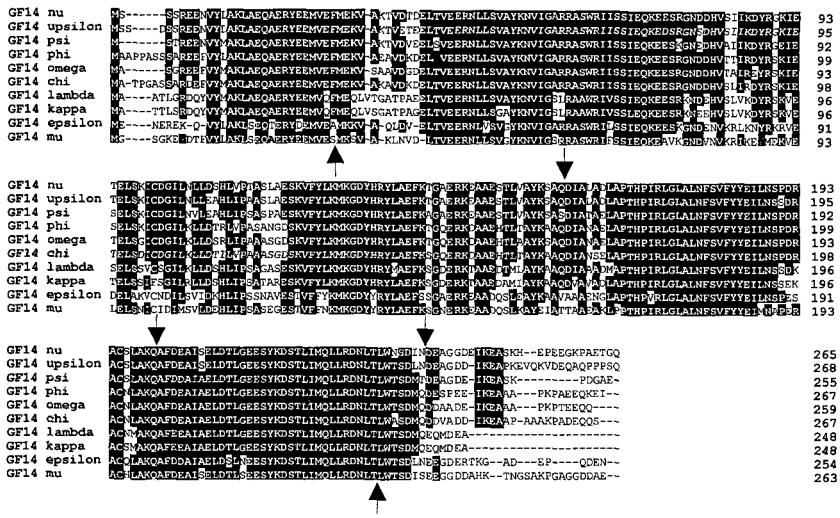

Figure 1. Alignment of the entire derived amino acid sequences of the Arabidopsis GF14 proteins. Dashes indicate gaps introduced to facilitate alignment. The numbers on the right indicate the serial numbers of the last residues at the end of GF14 amino acid sequences. The three downward arrows indicate the positions of introns identified in GRF1χ-GF14χ, GRF2ω-GF14ω, GRF3ψ-GF14ψ, GRF4ϕ-GF14ϕ, and GRF5υ-GF14υ gene sequences. The two upward arrows indicate the positions where the amino acid sequences were truncated to facilitate subsequent phylogenetic analysis.

five different restriction enzymes produced 7 to 15 bands that hybridized with the GF14ϕ probe (Fig. 3, lanes 1–5). Similar results were obtained using a probe made from a divergent GF14ϵ cDNA clone under the same conditions (Fig. 3, lanes 6–10). Many bands hybridizing to the ϵ probe are also recognized by the ϕ probe, indicating that these divergent probes cross-hybridize at this low stringency.

Evolution of 14-3-3 Sequences

An Ancient Split among Plant 14-3-3 Proteins

To study the evolution of 14-3-3 proteins, protein phylogenetic trees were initially generated based on the alignment of truncated Arabidopsis GF14 protein sequences in which highly variable parts of N and C termini were removed (Fig. 1). These regions are so divergent that proper alignment becomes problematic (Ferl et al., 1994). A similarly truncated 14-3-3-1 protein sequence from *Entamoeba histolytica* was also included in the alignment (data not shown) and later employed as an outgroup because *E. histolytica,* an amitochondrial protozoan, represents the most divergent species in terms of systematic classification.

As shown in Figure 4, A and B, unrooted networks were made using both neighbor-joining and protein parsimony methods from PHYLIP (Felsenstein, 1989), and only those branches that survived the bootstrap analysis at 80% are indicated. Both trees place GF14ϵ and GF14μ in one cluster, GF14κ and GF14λ in one cluster, and GF14ψ by itself. GF14ω, GF14χ, and GF14ϕ are also grouped in one cluster. The placement of GF14υ and GF14ν is the only variation in the two networks. Neighbor-joining (Fig. 4A) supports the grouping of GF14υ and GF14ν with a subtending branch, whereas parsimony (Fig. 4B) leaves GF14υ and GF14ν as independent basal branches. The grouping of GF14ω, GF14χ, and GF14ϕ is also supported by the fact that this group lacks the 5′ intron characteristic of GF14ψ and GF14υ.

To further characterize the evolutionary relationship of 14-3-3 proteins among different species (especially in plants), all available 14-3-3 protein sequences from plants and certain representative sequences from other organisms were retrieved from the protein database of GenBank (Table III), truncated to eliminate the highly divergent N and C termini, and aligned (Fig. 5). Trees were generated in a similar manner as described above. As shown in Figure 6,

Table II. *Percentage of amino acid and coding nucleotide identity of Arabidopsis GF14s*

Comparison was done by the GeneWorks program. Amino acid identity is shown in the lower part and nucleotide identity in the upper part of the matrix table.

	GF14ν	GF14υ	GF14ψ	GF14ϕ	GF14ω	GF14χ	GF14λ	GF14κ	GF14ε	GF14μ
							Nucleotide identity			
GF14ν		83	77	71	71	69	66	65	65	64
GF14υ	89		77	71	69	70	67	67	63	65
GF14ψ	84	84		72	71	71	67	66	65	65
GF14ϕ	80	78	79		78	84	68	67	68	64
GF14ω	82	79	81	89		79	67	67	63	64
GF14χ	79	77	77	87	89		67	69	60	63
GF14λ	69	68	69	71	72	71		86	61	62
GF14κ	69	68	69	68	70	69	92		61	62
GF14ε	66	65	69	65	64	63	63	62		68
GF14μ	67	66	67	64	66	64	60	60	70	
				Amino acid identity						

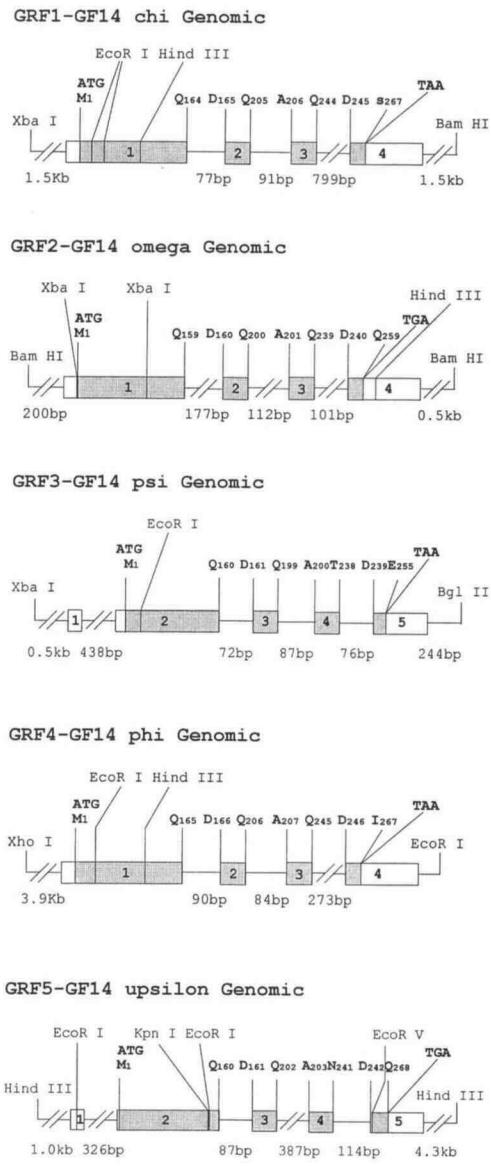

Figure 2. Physical structure of the GF14 genes. A restriction map of the insert of each genomic clone is shown at the top. Coding regions are shown by hatched blocks, and 5′ and 3′ untranslated regions are shown by open blocks. The 5′ and 3′ flanking sequences and introns are designated by lines. Numbers above the gene structures indicate the codon positions, and the numbers below show the sizes of the introns. The amino acid split by introns are labeled. Start and stop codons are also indicated.

both trees show similar but nonidentical topology, indicating that the major evolutionary groupings are correct, even though the relative branch positions of some ancient branches remain uncertain.

Most plant sequences (subgroup III) cluster separately from those of mammals (subgroup I) with a high degree of confidence in both trees, indicating that mammalian and plant 14-3-3 lineages have been on distinct and separate evolutionary tracts since their earliest divergence. This result is also confirmed by the significant divergence in genomic structure of plant and mammalian 14-3-3 genes. Similar conclusions were also reached in previous studies that were based on fewer sequences (Ferl et al., 1994; Wang and Shakes, 1996). Notable exceptions are the 14-3-3 isoforms indicated as subgroup II in Figure 6. This distinct group includes 14-3-3 proteins from both plants and mammals and the grouping is supported in both trees. The plant isoforms in this group include Arabidopsis GF14ϵ and GF14μ, tobacco (*Nicotiana tabacum*) CZ1, and *Vicia faba* 14-3-3b, and form a distinct grouping within the phylogenetic tree of plant 14-3-3 proteins. Arabidopsis GF14ϵ and GF14μ are more similar to tobacco CZ1 and *V. faba* 14-3-3b, respectively, than to other Arabidopsis homologs. The separation of these isoforms away from subgroups I and II is clearly supported by very strong bootstrap values (Fig. 6). Early branches in subgroup II are weakly supported by bootstrap analysis, indicating ancient branches from an as yet unresolved basal polychotomy.

At the amino acid sequence level, GF14ϵ and GF14μ represent the most divergent among all Arabidopsis isoforms, and are more identical to each other than to other homologs (Fig. 1; Table II). In addition, *E. coli*-expressed GF14ϵ and GF14μ displayed the weakest immunoreactivity with the monoclonal antibody relative to all other isoforms (data not shown). These data suggest that GF14ϵ, GF14μ, tobacco CZ1, and *V. faba* 14-3-3b all belong to a novel class of genes within the plant 14-3-3s, which is distinct from the class that contains all of the other plant sequences. The duplication event that led to the formation of these two ancient classes of plant 14-3-3 isoforms apparently predated the divergence of Arabidopsis, tobacco, and *V. faba*.

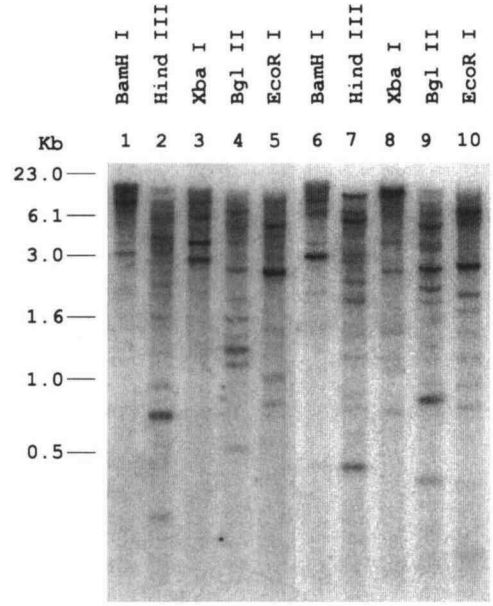

Figure 3. Genomic Southern-blot analysis reveals a large GF14 gene family. Arabidopsis genomic DNA (1.2 μg) was restricted with *Bam*HI, *Hin*dIII, *Xba*I, *Bgl*II, and *Eco*RI, resolved on a 0.8% agarose gel, blotted to a nylon membrane, and hybridized with a probe made from GF14ϕ (lanes 1–5) under the conditions described in "Materials and Methods." After exposure the same blot was stripped and hybridized with a probe made from GF14ϵ (lanes 6–10). Molecular mass (Kb) is indicated by numbers on the left.

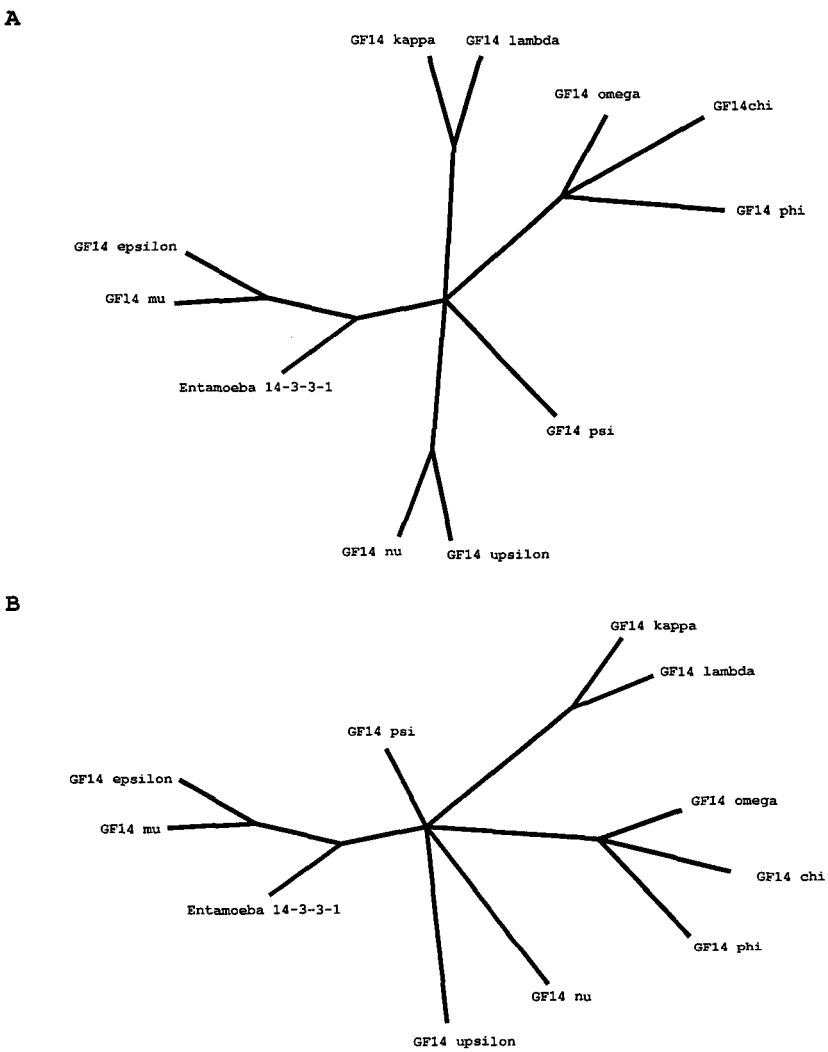

Figure 4. Unrooted neighbor-joining (A) and protein parsimony (B) network analysis of Arabidopsis GF14 proteins and *E. histolytica* 14-3-3-1. The branch lengths shown in the network are proportional to the branch lengths calculated by neighbor-joining and protein parsimony programs contained in the PHYLIP program package (version 3.5; Felsenstein, 1989). Only those branches that survived bootstrap analysis at 80% are indicated.

Plant 14-3-3s versus Those of Mammals

The tight clustering of non-ϵ plant 14-3-3 isoforms (subgroup III) in both trees indicates that most of the present-day isoform diversity arose after the separation of ϵ-like and non-ϵ isoforms through independent duplications within each subbranch. As shown in both trees, the non-ϵ isoforms from monocots appear to separate from those of dicots (Fig. 6). The branching patterns of some non-ϵ isoforms, such as those in Arabidopsis, indicate that recent duplication events were responsible for the generation of some isoforms. This is in contrast to the situation in mammals, in which various isoform groups were well established before the point of mammalian, and possibly vertebrate, divergence (Wang and Shakes, 1996).

DISCUSSION

Arabidopsis Contains a Complex 14-3-3 Gene Family

The 10 Arabidopsis 14-3-3 genes identified and characterized here represent, to our knowledge, the largest 14-3-3 gene family in any species examined to date. An exhaustive search of the latest Arabidopsis dbEST (with more than 25,000 entries) using all 10 14-3-3 sequences retrieved only EST sequences that were identical to the probes (data not shown). It is highly likely that these 10 distinct sequences represent all of the members of the 14-3-3 protein family in Arabidopsis. Initial results from northern-blot analyses of Arabidopsis suspension cells (data not shown) and the presence of all 10 unique sequences in the dbEST indicate that they are all expressed in Arabidopsis at the mRNA level. In spite of their high conservation of amino acid sequences, different Arabidopsis 14-3-3 isoforms maintain their distinctiveness, especially at their N- and C-terminal ends (Fig. 1), suggesting that they may encode for different functions.

Evolution of Plant 14-3-3s

Plant 14-3-3 proteins are divided into two main divergent classes, ϵ-like and non-ϵ isoforms. Arabidopsis GF14ϵ, GF14μ, tobacco CZ1, and *V. faba* 14-3-3b belong to ϵ-like isoforms, whereas the rest of the plant 14-3-3s belong to non-ϵ isoforms. Because of the lack of sequence data from

Table III. *14-3-3 sequences in this study*

Organism	Gene Type	Accession No.	Reference
Plant			
A. thaliana	GF14ω	M96855	Lu et al., 1992
A. thaliana	GF14χ	L09112	Lu et al., 1994
A. thaliana	GF14ϕ	L09111	Lu et al., 1994
A. thaliana	GF14ψ	L09110	Lu et al., 1994
A. thaliana	GF14υ	L09109	Lu et al., 1994
A. thaliana	GF14ε	U36446	This report
A. thaliana	GF14κ	U36447	This report
A. thaliana	GF14λ	U68545	This report
A. thaliana	GF14μ	U60444	This report
A. thaliana	GF14ν	U60445	This report
Hordeum vulgare (barley)	Hv14-3-3a	X62388	Brandt et al., 1992
H. vulgare	Hv 14-3-3b	X93170	Brandt et al., 1992
N. tabacum (tabacco)	CZI	S76737	Chen et al., 1994
Oenothera hookeri		X62838	Hirsch et al., 1992
Oryza sativa (rice)	S94	D16140	Kidou et al., 1993
O. sativa	GF14-b	U65956	T.F. Schultz, J. Davis, and R.S. Quatrano, unpublished data
O. sativa	GF14-c	U65957	T.F. Schultz, J. Davis, and R.S. Quatrano, unpublished data
O. sativa	GF14-d	U65958	T.F. Schultz, J. Davis, and R.S. Quatrano, unpublished data
Pisum sativum (pea)		U15036	Stankovic et al., 1995
Solanum lycopersicum (tomato)	GF14-T3	L29151	Laughner et al., 1994
S. lycopersicum	GF14-T4	L29150	Laughner et al., 1994
S. tuberosum (potato)		X97724	G. Wilcczynski and J. Szopa, unpublished data
S. tuberosum (potato root)		X87370	G. Wilcczynski and J. Szopa, unpublished data
V. faba	Vfa-1433a	Z48504	G. Saalbach, V. Christov, and M. Schwerdel, unpublished data
V. faba	Vfa-1433b	Z48505	G. Saalbach, V. Christov, and M. Schwerdel, unpublished data
Z. mays (maize)	GF14-12	M96856	de Vetten et al., 1992
Yeast			
S. cerevisiae	BMH1	X66206	van Heusden et al., 1992
S. cerevisiae	BMH2	x84817	van Heusden et al., 1995
Mammal			
Homo sapiens (human)	ε	U54778	Chong et al., 1996
Rattus norvegicus (rat)	β	S55223	Watanabe et al., 1993
R. norvegicus	ε	M84416	Roseboom et al., 1994
R. norvegicus	η	D17445	Watanabe et al., 1991
R. norvegicus	γ	S55305	Watanabe et al., 1993
Amoeba			
E. histolytica	14-3-3-1	U13418	J. Samuelson, P. Shen, G. Meckler, S. Descoteaux, H. Fu, and A. Lohia, unpublished data

monocot species, it is still unclear whether the split that generated ϵ-like and non-ϵ isoforms occurred before the separation of monocots and dicots. However, one intriguing observation is that in rice there is a family of 14-3-3 homologs similar to that of Arabidopsis. Thus, it is very likely that there are Arabidopsis ϵ-like isoforms in rice as well as in other monocots.

The deep branching pattern of plant ϵ-like isoforms from non-ϵ isoforms suggests that the split that produced these two lineages might have occurred during the very earliest divergence of ancient plant species (Fig. 6). The divergence between ϵ-like and non-ϵ isoforms was further demonstrated by the results from detailed analyses of DNA and protein sequences, dimerization domain mapping (Wu et al., 1997), and immunoreactivity of expressed Arabidopsis 14-3-3 proteins (data not shown).

The evolutionary relationship between plant ϵ-like and mammalian ϵ isoforms remains to be fully investigated. Mammalian ϵ is the most unique isoform of all mammalian 14-3-3 proteins (Ferl et al., 1994; Wang and Shakes, 1996); it is more similar to plant and yeast 14-3-3 isoforms than to those of other mammals, and was thought to serve as a functionally conserved copy of the original ancestral 14-3-3 genes (Wang and Shakes, 1996). Based on the ancient divergence observed in the current study, the Arabidopsis ϵ-like 14-3-3 proteins may similarly serve ancient and essential functions different from those of the non-ϵ plant isoforms.

In Arabidopsis some non-ϵ isoforms seem to arise from recent duplication events. It is still unclear how many non-ϵ isoforms were generated after the divergence of different species. Isolation and analysis of 14-3-3 proteins from additional plant species will provide a better picture of exactly when the latter duplication events occurred.

Plant 14-3-3 Functions

All phylogenetic analyses support early and clear divergence of plant non-ϵ and mammal lineages. Therefore, there can be no specialized functions shared among the

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Rat eta                 MKAV------TELNEPLSNEDRNLLSVAYKNVVGARRSSWRVISSIEQKTMADGNEKK-LEKVKAYREKIEKELETVCNDVLALLDKFLIKNCNDFQYESKVFYLKMKGD 103
Rat gamma               MKNV------TELNEPLSNEERNLLSVAYKNVVGARRSSWRVISSIEQKTSADGNEKK-IEMVRAYREKIEKELEAVCQDVLSLLDNYLIKNCSETQYESKVFYLKMKGD 103
Rat beta                MKAV------TEQGHELSNEERNLLSVAYKNVVGARRSSWRVISSIEQKTERN--EKK-QQMGKEYREKIEAELQDICSDVLELLDKYLILNATHA--ESKVFYLKMKGD 99
At GF14 lambda          MEQL---VTGATPAEELTVEERNLLSVAYKNVIGSLRAAWRIVSSIEQKEESRKNDEH-VSLVKDYRSKVESELSSVCSGILKLLDSHLIPSAGAS--ESKVFYLKMKGD 104
At GF14 kappa           MEQL---VSGATPAGELTVEERNLLSGAYKNVIGSLRAAWRIVSSLEQKEESRKNEEH-VSLVKDYRSKVETELSSIFSGILRLLDSHLIPSATAR--ESKVFYLKMKGD 104
Potato                  MEKV-----AKVDVEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNEDH-VSSIKEYRVKIEAELSKICDGILSLLESHLVPSASTA--ESKVFYLKMKGD 102
At GF14 mu              MKSVAKL-----NV-DLTVEERNLLSVGYKNVIGSRRASWRIFSSIEQKEAVKGNDVN-VKRIKEYMEKVELELSNICIDIMSVLDEHLIPSASEG--ESTVFFNKMKGD 101
Vfa-1433b               MKNVANL-----DV-ELTIEERNLLSVGYKNVIGARRASWRILSSIEQKEESKGNDVN-AKRIKEYRHKVETELSNICIDVMRVIDEHLIPSAAAG--ESTVFYYKMKGD 101
Tobacco CZ1             MKTVAKM-----DV-ELTVEERNLVSVGYKNVIGARRASWRILSSIEQKEESKGHDQN-VKRIKTYQQRVEDELTKYAL-TLSVIDEHVVPSSTSG--ESTVFYYKMKGD 100
At GF14 epsilon         MKKVAQL-----DV-ELTVEERNLVSVGYKNVIGARRASWRILSSIEQKEESKGNDEN-VKRLKNYRKRVEDELAKVCNDILSVIDKHLIPSSNAV--ESTVFFYKMKGD 101
Rat eps                 MKKVAGM-----DV-ELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEENKGGEDK-LKMIREYRQMVETELKLICCDILDVLDKHLIPAANTG--ESKVFYYKMKGD 101
human epsilon           MKKVAGM-----DV-ELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEENKGGEDK-LKMIREYRQMVETELKLICCDILDVLDKHLIPAANTG--ESKVFYYKMKGD 101
Pea                     MEKVSANA----DSEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNEDH-VAVIRDYRSKIESELSNICDGILKLLDTRLIPSASSG--DSKVFYLKMKGD 103
Vfa-1433a               MEKVTAAV----ESEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNDEH-VSVIRDYRSKIETELSNICNGILKLLDSRLIPSAALG--DSKVFYLKMKGD 103
At GF14 omega           MEKVSAAV----DGDELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNDDH-VTAIREYRSKIETELSGICDGILKLLDSRLIPAAASG--DSKVFYLKMKGD 103
oenothera               MEKVCAAA----DSEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNDDH-VSTIRDYRSKIETELSNICGGILKLLDSRLIPSAASG--DSKVFYLKMKGD 103
At GF14 phi             MEKVAEAV----DKDELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNDDH-VTTIRDYRSKIESELSKICDGILKLLDTRLVPASANG--DSKVFYLKMKGD 103
At GF14 chi             MEKVAKAV----DKDELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNDDH-VSLIRDYRSKIETELSDICDGILKLLDTILVPAAASG--DSKVFYLKMKGD 103
Tomato GF14-T3          MEKVSNSL----GSEELTVEERNLLSVAYKNVIGARRASWRIISSVEQKEESRGNEEH-VNSIREYRSKIENELSKICDGILKLLDSKLIPSATSG--DSKVFYLKMKGD 103
Potato root             MEKVVAAL----DT-ELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNEDH-VASIKKYRSQIENELTSICNGILKLLDSKLIGSAATG--DSKVFYLKMKGD 102
Barley 14-3-3a          MEKVAKTA----DVGELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNEAY-VASIKEYRTRIETELSKICDGILKLLDSHLVPSATAA--ESKVFYLKMKGD 103
Rice s94                MEKVAKTT----DVGELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNEAY-VASIKEYRSRIETELSKICDGILKLLDSHLVPSATAA--ESNVFYLKMKGD 103
Tomato GF14-T4          MEKVAKTA----DVEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNEDH-VNTIKEYRSKIEADLSKICDGILSLLESNLIPSASTA--ESKVFHLKMKGD 103
Rice GF14d              MERVARAAGGASGGEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEEGRGNDAH-AATIRSYRGKIEAELARICDGILALLDSHLVPSAGAA--ESKVFYLKMKGD 107
At GF14 nu              MEKVAKTV----DTDELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNDDH-VSIIKDYRGKIETELSKICDGILNLLDSHLVPTASLA--ESKVFYLKMKGD 103
At GF14 upsilon         MEKVAKTV----ETEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEDSRGNSDH-VSIIKDYRGKIETELSKICDGILNLLEAHLIPAASLA--ESKVFYLKMKGD 103
At GF14 psi             MEKVAKTV----DVEELSVEERNLLSVAYKNVIGARRASWRIISSIEQKEESKGNEDH-VAIIKDYRGEIESELSKICDGILNVLEAHLIPSASPA--ESKVFYLKMKGD 103
Barley 14-3-3b          MEKVAKTV----DSEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNEDR-VTLIKDYRGKIEVELTKICDGILKLLDSHLVPSSTAP--ESKVFYLKMKGD 103
Rice GF14b              MEKVAKTV----DSEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEESRGNEDR-VTLIKDYRGKIETELTKICDGILKLLESHLVPSSTAP--ESKVFYLKMKGD 103
Maize GF14-12           MEKVAKTV----DSEELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEEGRGNEDR-VTLIKDYRGKIETELTKICDGILKLLESHLVPSSTAP--ESKVFYLKMKGD 103
Rice GF14c              MEKVAKTV----DVEELTVEERNLLSVAYKNVIGARRASWRIVSSIEQKEEGRGNEEH-VTLIKEYRGKIEAELSKICDGILKLLDSHLVPSSTAA--ESKVFYLKMKGD 103
Sc BMH1                 MKTVAS------SGQELSVEERNLLSVAYKNVIGARRASWRIVSSIEQKEESKEKSEHQVELICSYRSKIETELTKISDDILSVLDSHLIPSATTG--ESKVFYYKMKGD 102
Sc BMH2                 MKAVAS------SGQELSVEERNLLSVAYKNVIGARRASWRIVSSIEQKEESKEKSEHQVELIRSYRSKIETELTKISDDILSVLDSHLIPSATTG--ECKVFYYKMKGD 102
Entamoeba 14-3-3-1      MKQVAEM-----EA-ELSIEERNLLSVAYKNVIGAKRASWRIISSLEQKEQAKGNDKH-VEIIKGYRAKIEKELSTCCDDVLKVIQENLLPKASTS--ESKVFFKKMEGD 101

Rat eta                 YYRYLAEVASGEKKN-SVVEASEAAYKEAFEISKEHMQPTHPIRLGLALNFSVFYYEIQNAPEQACLLAKQAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLT 205
Rat gamma               YYRYLAEVATGEKRA-TVVESSEKAYSEAHEISKEHMQPTHPIRLGLALNYSVFYYEIQNAPEQACHLAKTAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLT 205
Rat beta                YFRYLSEVASGDNKQ-TTVSNSQQAYQEAFEISKKEMQPTHPIRLGLALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTLNEESYKDSTLIMQLLRDNLT 201
At GF14 lambda          YHRYMAEFKSGD-ERKTAAEDTMLAYKAAQDIAAADMAPTHPIRLGLALNFSVFYYEILNSSDKACNMAKQAFEEAIAELDTLGEESYKDSTLIMQLLRDNLT 206
At GF14 kappa           YHRYLAEFKSGD-ERKTAAEDAMIAYKAAQDVAVADLAPTHPIRLGLALNFSVFYYEILNSSEKACSMAKQAFEEAIAELDTLGEESYKDSTLIMQLLRDNLT 206
Potato                  YHRYLAEFKTGARERKEAAENTLLAYKSAQDIALAELTPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDDAIAELDTLGEESYKDSTLIMQLLRDNLT 205
At GF14 mu              YYRYLAEFKSGNERKEAA-DQSLKAYEIATTAAEAKLPPTHPIRLGLALNFSVFYYEIMNAPERACHLAKQAFDEAISELDTLSEESYKDSTLIMQLLRDNLT 203
Vfb-1433b               YYRYLAEFKTGNEKKEAG-DQSMKAYESATTAAEAELPPTHPIRLGLALNFSVFYYEILNSPERACHLAKQAFDEAISELDTLNEESYKDSTLIMQLLRDNLT 203
Tobacco CZ1             YYRYLAEFKSGDDRKEAA-DQSLKAYEAATATASADLAPTHPIRLGLALNFSVFYYEILNSPERACHLAKQAFDEAIAELDSLSEESYKDSTLIMQLLRDNFT 202
At GF14 epsilon         YYRYLAEFSSGAERKEAA-DQSLEAYKAAVAAAENGLAPTHPVRLGLALNFSVFYYEILNSPESACQLAKQAFDDAIAELDSLNEESYKDSTLIMQLLRDNLT 203
Rat epsilon             YHRYLAEFATGNDRKEAA-ENSLVAYKAASDIAMTELPPTHPIRLGLALNFSVFYYEILNSPDRACRLAKAAFDDAIAELDTLSEESYKDSTLIMQLLRDNLT 203
Human epsilon           YHRYLAEFATGNDRKEAA-ENSLVAYKAASDIAMTELPPTHPIRLGLALNFSVFYYEILNSPDRACRLAKAAFDDAIAELDTLSEESYKDSTLIMQLLRDNLT 203
Pea                     YHRYLAEFKTGAERKEAA-ESTLTGYKSAQDIANAELPPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAIAELDTLGEESYKDSTLIMQLLRDNLT 205
Vfa-1433a               YHRYLAEFKSGAERKDAA-ESTLTAYKSAQDIANTELPPTHPIRLGLALNFSVFYYEILNSPDRACGLAKQAFDEAIAELDTLGEESYKDSTLIMQLLRDNLT 205
At GF14 omega           YHRYLAEFKTGQERKDAA-EHTLAAYKSAQDIANAELAPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAIAELDTLGEESYKDSTLIMQLLRDNLT 205
Oenothera               YHRYLAEFKTGAERKEAA-ESTLSAYKAAQDIANAELAPTHPIRLGLALNFSVFYYEILNSPDRACNLANEAFDEAIAELDTLEEESYKDSTLIMQLLRDNLT 205
At GF14 phi             YHRYLAEFKTGQERKDAA-EHTLTAYKAAQDIANAELAPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAIAELDTLGEESYKDSTLIMQLLRDNLT 205
AT GF14 chi             YHRYLAEFKSGQERKDAA-EHTLTAYKAAQDIANSELAPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAIAELDTLGEESYKDSTLIMQLLRDNLT 205
Tomato GF14-T3          YHRYLAEFKTGAERKEAA-ESTLTGYKAAQDIASAELAPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAIAELDTLGEESYKDSTLIMQLLRDNLT 205
Potato root             YYRYLAEFKTGTERKEAA-ENTLSAYKSAQDIANGELAPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAIADVDTLGEESYKDSTLIMQLLRDNLT 204
Barley 14-3-3a          YHRYLAEFKAGAERKEAA-ENTLVAYKSAQDIALADLPTTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAIAELDSLGEESYKDSTLIMQLLRDNLT 205
Rice s94                YHRYLAEFKSGAERKEAA-ENTLVAYKSAQDIALADLPTTHPIRLGLALNLSVFYYEILNSPDRACNLAKQAFDDAIAELDTLGEESYKDSTLIMQLLRDNLT 205
Tomato GF14-T4          YHRYLAEFKTGTERKEAA-ENTLLAYKSAQDIALAELAPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLT 205
Rice GF14d              YHRYLAEFKSGDERKQAA-ESTMNAYKAAQDIALADLAPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDSLGEESYKDSTLIMQLLRDNLT 209
At GF14 nu              YHRYLAEFKTGAERKEAA-ESTLVAYKSAQDIALADLAPTHPIRLGLALNFSVFYYEILNSPDRACSLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLT 205
At GF14 upsilon         YHRYLAEFKTGAERKEAA-ESTLVAYKSAQDIALADLAPTHPIRLGLALNFSVFYYEILNSSDRACSLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLT 205
At GF14 psi             YHRYLAEFKAGAERKEAA-ESTLVAYKSASDIATAELAPTHPIRLGLALNFSVFYYEILNSPDRACSLAKQAFDDAIAELDTLGEESYKDSTLIMQLLRDNLT 205
Barley 14-3-3b          YYRYLAEFKSGTERKDAA-ENTMVAYKAAQEIALAELPPTHPIRLGLALNFSVFYYEILNSPDRACDLAKQAFDEAISELDSLSEESYKDSTLIMQLLRDNLT 205
Rice GF14b              YYRYLAEFKTGAERKDAA-ENTMVAYKAAQDIALAELPPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDTLSEESYKDSTLIMQLLRDNLT 205
Maize GF14-12           YYRYLAEFKTGAERKDAA-ENTMVAYKAAQDIALAELAPTHPIRLGLALNFSVFYYEILNSPDRACSLAKQAFDEAISELDTLSEESYKDSTLIMQLLHDNLT 205
Rice GF14c              YHRYLAEFKTGAERKEAA-ESTMVAYKAAQDIALADLAPTHPIRLGLALNFSVFYYEILNSPDKACNLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLT 205
Sc BMH1                 YHRYLAEFSSGDARKAT-NASLEAYKTASEIATTELPPTHPIRLGLALNFSVFYYEIQNSPDKACHLRKQAFDDAIAELDTLSEESYKDSTLIMQLLRDNLT 204
Sc BMH2                 YHRYLAEFSSGDARKAT-NSSLEAYKTASEIATTELPPTHPIRLFLALNFSVFYYEIQNSPDKACHLAKQAFDDAIAELDTLSEESYKDSTLIMQLLRDNLT 204
Entamoeba 14-3-3-1      YYRYFAEFTVDEKRKEVA-DKSLAAYTEATEISNAELAPTHPIRLGLALNFSVFYFEIMNDADKACQLAKQAFDDAIAKLDEVPENMYKDSTLIMQLLRDNLT 203
```

Figure 5. Alignment of truncated 14-3-3 homolog sequences. The sequences derived from those listed in Table III were truncated at conserved residues to facilitate an unambiguous alignment.

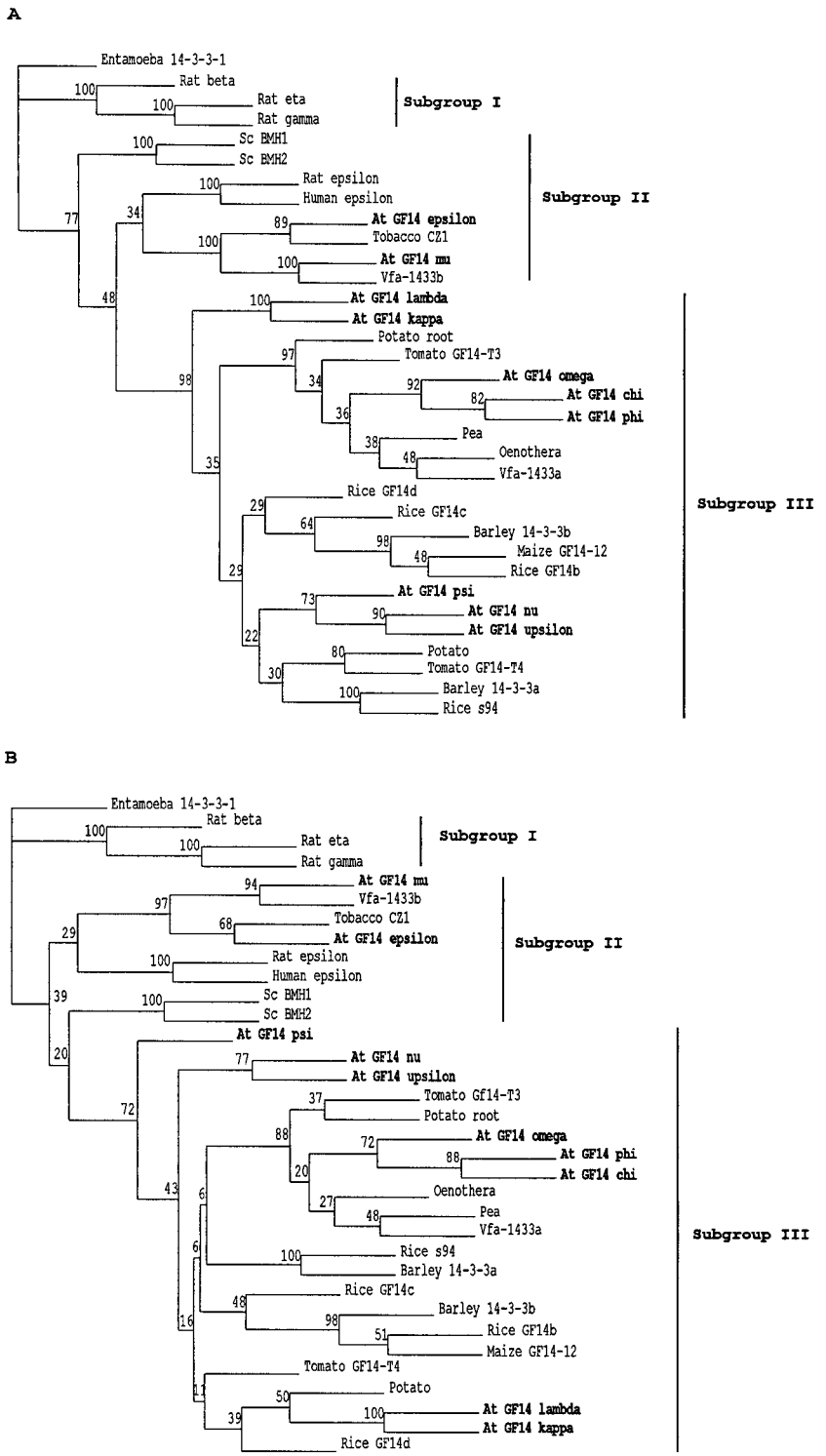

Figure 6. Bootstrap majority-rule consensus trees derived from 100 neighbor-joining (A) and protein parsimony (B) replicates with alignment shown in Figure 5.

known mammalian and plant isoforms, except perhaps the ϵ isoforms. Fundamental properties can be shared, but specialized functions cannot be shared by animal and plant isoforms as a result of evolutionary heritage. Evidence supporting this theory has been emerging since the early stages of plant 14-3-3 research. Specialized functions and activities that are as yet unique to plants include association with a DNA/protein complex (de Vetten et al., 1992; Lu et al., 1992), receptors for fusicoccin (Korthout and de Boer, 1994; Oecking et al., 1994), and binding and regulation of nitrate reductase (Bachmann et al., 1996a, 1996b; Moorhead et al., 1996).

Fundamental properties and activities shared among animals and plants include phosphoprotein-binding activities

and participation in signaling pathways. The evidence for shared functions includes observations that an Arabidopsis 14-3-3 isoform can perform the functions of yeast and mammalian isoforms (Lu et al., 1994), that several plant isoforms are able to regulate the protein kinase C activities such as those from mammals (Chen et al., 1994; Lu et al., 1994), and that expressed Arabidopsis GF14 proteins bind to nitrate reductase (Bachmann et al., 1996a, 1996b; Moorhead et al., 1996), which contains a phosphoserine consensus sequence known to be bound by 14-3-3 proteins in mammals (Muslin et al., 1996). Therefore, the next question about the diversity of 14-3-3 isoforms would be how different isoforms with similar fundamental properties carry out different or specific functions. One answer may be that different isoforms exhibit subtle quantitative or qualitative alterations in these fundamental properties (Bachmann et al., 1996a) that result in the appearance of specificity.

Another answer may lie in the intrinsic characteristics of different 14-3-3 genes that determine their distinct expression patterns and their different abilities to respond to differential external stimuli. The characterized 14-3-3 isoforms in Arabidopsis, GF14χ, GF14ψ, GF14ω, GF14υ, and GF14ϵ, display a certain degree of cell- and organ-specific expression patterns (Lu et al., 1992; Daugherty et al., 1996; K. Wu and R.J. Ferl, unpublished observations). In addition, environmental stimuli influence 14-3-3 expression in plants.

Some 14-3-3 isoforms were demonstrated to be induced by low temperature (Jarillo et al., 1994) and hypoxia (de Vetten and Ferl, 1995), down-regulated during acclimation to high salt (Chen et al., 1994), regulated by both salt and low temperature (Kidou et al., 1993), and induced after pathogen attack (Brandt et al., 1992). These observations suggest the involvement of specific 14-3-3s in specific signal transduction pathways, and distinct response profiles of various isoforms may simply serve to ensure expression of the fundamental activities during times of need.

The identification and initial characterization of the 10 Arabidopsis 14-3-3 isoforms presents us with an unprecedented opportunity to fully understand the fundamental function(s) and diversity of 14-3-3 proteins within a single organism. Characterization of the biochemical properties of each isoform will lend further insight into their potential roles. Defining the phenotype of transgenic plants with antisense genes of different isoforms will contribute to the clarification of the pathways affected by 14-3-3 members. Together with an appreciation of the localization profiles of all the isoforms, we may gain a greater understanding of the entire 14-3-3 gene family in Arabidopsis and in other organisms.

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LITERATURE CITED

Aitken A (1995) 14-3-3 proteins on the MAP. Trends Biochem Sci **20:** 95–97

Alam R, Hachiya N, Sakaguchi M, Kawabata S, Iwanago S, Kitajima M, Mihara K, Omura T (1994) cDNA cloning and characterization of mitochondrial import stimulation factor (MSF) purified from rat liver cytosol. Biochem J **116:** 416–425

Bachmann M, Huber JL, Athwal GS, Wu K, Ferl RJ, Huber SC (1996a) 14-3-3 proteins associate with the regulatory phosphorylation site of spinach leaf nitrate reductase in an isoform-specific manner and reduce dephosphorylation of Ser-543 by endogenous protein phosphatases. FEBS Lett **398:** 26–30

Bachmann M, Huber JL, Liao PC, Gage DA, Huber SC (1996b) The inhibitor protein of phosphorylated nitrate reductase from spinach (*Spinacia oleracea*) leaves is a 14-3-3 protein. FEBS Lett **387:** 127–131

Brandt J, Thordal-Christensen H, Vad K, Gregersen PL, Collinge DB (1992) A pathogen-induced gene of barley encodes a protein showing high similarity to a protein kinase regulator. Plant J **2:** 815–820

Chen Z, Fu H, Liu D, Chang PL, Narasimhan M, Ferl RJ, Hasegawa PM, Bressan RA (1994) A NaCl-regulated plant gene encoding a brain protein homolog that activates ADP ribosyltransferase and inhibits protein kinase C. Plant J **5:** 729–740

Chong SS, Tanigami A, Roschke AV, Ledbetter DH (1996) 14-3-3 epsilon has no homology to LIS1 and lies telomeric to it on chromosome 17p13.3 outside the Miller-Dieker syndrome chromosome region. Genome Res **6:** 735–741

Conklin DS, Galaktionov K, Beahc D (1995) 14-3-3 proteins associate with cdc25 phosphatases. Proc Natl Acad Sci USA **92:** 7892–7896

Daugherty CJ, Rooney MF, Miller PW, Ferl RJ (1996) Molecular organization and tissue-specific expression of an Arabidopsis 14-3-3 gene. Plant Cell **8:** 1239–1248

de Vetten NC, Ferl RJ (1994) Two genes encoding GF14 (14-3-3) proteins in *Zea mays*. Plant Physiol **106:** 1593–1604

de Vetten NC, Ferl RJ (1995) Transcriptional regulation of environmentally inducible genes in plants by an evolutionarily conserved family of G-box binding factors. Intl J Biochem **26:** 1055–1068

de Vetten NC, Lu G, Ferl RJ (1992) A maize protein associated with the G-box binding complex has homology to brain regulatory proteins. Plant Cell **4:** 1295–1307

Fantl WJ, Muslin AJ, Kikuchi A, Martin JA, MacNicol AM, Gross RW, Williams LT (1994) Activation of Raf-1 by 14-3-3 proteins. Nature **371:** 612–613

Felsenstein J (1989) PHYLIP: Phylogeny Inference Package, Version 3.2. Cladistics **5:** 164–166

Ferl RJ (1996) 14-3-3 proteins and signal transduction. Annu Rev Plant Physiol Plant Mol Biol **47:** 49–73

Ferl RJ, Lu G, Bowen BW (1994) Evolutionary implications of the family of 14-3-3 brain protein homologs in *Arabidopsis thaliana*. Genetica **92:** 129–138

Ferl RJ, Nick HS (1987) In vivo detection of regulatory factor binding sites in the 5′ flanking region of maize Adh1. J Biol Chem **262:** 7947–7950

Ford JC, Al-Khodairy F, Fotou E, Sheldrick KS, Griffiths DJF, Carr AM (1994) 14-3-3 protein homologs required for the DNA damage checkpoint in fission yeast. Science **265:** 533–537

Freed E, Symons M, Macdonald SG, McCormick F, Ruggieri R (1994) Binding of 14-3-3 proteins to the protein kinase Raf and effects on its activation. Science **265:** 1713–1716

Fu H, Xia K, Pallas DC, Cui C, Conroy K, Narsimhan RP, Mamon H, Collier RJ, Roberts TM (1994) Interaction of the protein kinase Raf-1 with 14-3-3 proteins. Science **266:** 126–133

Hirsch S, Aitken A, Bertsch U, Soll J (1992) A plant homologue to mammalian brain 14-3-3 protein and protein kinase C inhibitor. FEBS Lett **296:** 222–224

Ichimura T, Isobe T, Yamauchi T, Fujisawa H (1987) Brain 14-3-3 protein is an activator protein that activates tryptophan

5-monooxygenase in the presence of Ca^{2+}, calmodulin-dependent protein kinase II. FEBS Lett **219:** 79–82

Irie K, Gotoh Y, Yashar BM, Errede B, Nishida E, Matsumoto K (1994) Stimulatory effects of yeast and mammalian 14-3-3 proteins on the Raf protein kinase. Science **265:** 1716–1719

Jarillo JA, Capel J, Leyva A, Martinez-Zapater JM, Salinas J (1994) Two related low-temperature-inducible genes of Arabidopsis encode proteins showing high homology to 14-3-3 proteins, a family of putative kinase regulators. Plant Mol Biol **25:** 693–704

Kidou S, Umeda M, Kato A, Uchimiya H (1993) Isolation and characterization of a rice cDNA similar to the bovine brain-specific 14-3-3 protein gene. Plant Mol Biol **21:** 191–194

Korthout HAAJ, de Boer AH (1994) A fusicoccin binding protein belongs to the family of 14-3-3 brain protein homologs. Plant Cell **6:** 1681–1692

Laughner B, Lawrence SD, Ferl RJ (1994) Two tomato fruit homologs of 14-3-3 mammalian brain proteins. Plant Physiol **105:** 1457–1458

Lu G, DeLisle AJ, de Vetten NC, Ferl RJ (1992) Brain proteins in plants: an *Arabidopsis* homolog to neurotransmitter pathway activators in part of a DNA binding complex. Proc Natl Acad Sci USA **89:** 11490–11494

Lu G, de Vetten NC, Sehnke PC, Isobe T, Ichimura T, Fu H, van Heusden GPH, Ferl RJ (1994) A single *Arabidopsis* GF14 isoform possesses biochemical characteristics of diverse 14-3-3 homologues. Plant Mol Biol **25:** 659–667

Lu G, Rooney MF, Wu K, Ferl RJ (1994) Five cDNAs encoding Arabidopsis GF14 proteins. Plant Physiol **105:** 1459–1460

Moore BW, Perez VJ (1967) Specific acidic proteins of the nervous system. *In* FD Carlson, ed, Physiological and Biochemical Aspects of Nervous Integration. Prentice-Hall, Englewood Cliffs, NJ, pp 343–359

Moorhead G, Douglas P, Morrice N, Scarabel M, Aitken A, MacKintosh C (1996) Phosphorylated nitrate reductase from spinach leaves is inhibited by 14-3-3 proteins and activated by fusicoccin. Curr Biol **6:** 1104–1113

Morgan A, Burgoyne RD (1992) Exo1 and Exo2 proteins stimulate calcium-dependent exocytosis in permeabilized adrenal chromaffin cells. Nature **355:** 833–836

Muratake T, Hayashi S, Ichikawa T, Kumanishi T, Ichimura Y, Kuwano R, Isobe T, Wang Y, Minoshima S, Shimizu N, Takahashi Y (1996) Structural organization and chromosomal assignment of the human 14-3-3 eta chain gene (YWHAH). Genomics **36:** 63–69

Muslin AJ, Tanner JW, Allen PM, Shaw AS (1996) Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphorerin. Cell **84:** 889–897

Nielsen PJ (1991) Primary structure of a human protein kinase regulator protein. Biochim Biophys Acta **1088:** 425–428

Oecking C, Eckerskorn C, Weiler EW (1994) The fusicoccin receptor of plants is a member of the 14-3-3 superfamily of eukaryotic regulatory proteins. FEBS Lett **352:** 163–166

Pallas DC, Fu H, Haehnel LC, Weller W, Collier RJ, Roberts TM (1994) Association of polyomavirus middle tumor antigen with 14-3-3 proteins. Science **265:** 535–537

Reuther GW, Fu H, Cripe LD, Collier RJ, Pendergast AM (1994) Association of the protein kinases c-Bcr and Bcr-Abl with proteins of the 14-3-3 family. Science **266:** 129–133

Rooney MF, Ferl RJ (1995) Sequences of three Arabidopsis general regulatory factor genes encoding GF14 (14-3-3) proteins. Plant Physiol **107:** 283–284

Roseboom PH, Weller JL, Babila T, Aitken A, Sellers LA, Moffett JR, Namboodiri MAA, Klein DC (1994) Cloning and characterization of the zeta and epsilon isoforms of the 14-3-3 proteins. DNA Cell Biol **13:** 629–640

Stankovic B, Garic-Stankovic A, Smith CM, Davies E (1995) Isolation, sequencing, and analysis of a 14-3-3 brain protein homolog from pea (*Pisum sativum* L.). Plant Physiol **107:** 1481–1482

Tanji M, Horwitz R, Rosenfeld G, Waymire JC (1994) Activation of protein kinase C by purified bovine brain 14-3-3s: comparison with tyrosine hydroxylase activation. J Neurochem **63:** 1908–1916

Toker A, Sellers LA, Amess B, Patel Y, Harris A, Aitken A (1992) Multiple isoforms of a protein kinase C inhibitor (KCIP-1/14-3-3) from sheep brain. Eur J Biochem **206:** 453–461

van Heusden GPH, Griffiths DJF, Ford JC, Chin-A-Woeng TFC, Schrader PAT, Carr AM, Steensma HY (1995) The 14-3-3 proteins encoded by the BMH1 and BMH2 genes are essential in the yeast *Saccharomyces cerevisiae* and can be replaced by a plant homologue. Eur J Biochem **229:** 45–53

van Heusden GPH, Wenzel TJ, Lagendijk EL, de Steensma HY, van den Berg JA (1992) Characterization of the yeast BMH1 gene encoding a putative protein homologous to mammalian protein kinase II activators and protein kinase C inhibitors. FEBS Lett **302:** 145–150

Wang W, Shakes DC (1996) Molecular evolution of the 14-3-3 protein family. J Mol Evol **43:** 384–398

Watanabe M, Isobe T, Ichimura T, Kuwano R, Takahashi Y, Kondo H (1993) Molecular cloning of rat cDNAs for beta and gamma subtypes of 14-3-3 protein and developmental changes in expression of their mRNAs in the nervous system. Mol Brain Res **17:** 135–146

Watanabe M, Isobe T, Okuyama T, Ichimura T, Kuwano R, Takahashi Y, Kondo H (1991) Molecular cloning of cDNA to rat 14-3-3 eta chain polypeptide and the neuronal expression of the mRNA in central nervous system. Brain Res Mol Brain Res **10:** 151–158

Wu K, Lu GH, Sehnke PC, Ferl RJ (1997) The heterologous interaction among plant 14-3-3 proteins and identification of regions that are important for dimerization. Arch Biochem Biophys **339:** 2–8

Yamauchi T, Nagata H, Fujisawa H (1981) A new activator protein that activates tryptophan 5-monooxygenase and tyrosine monooxygenase in the presence of Ca^{2+}-calmodulin-dependent protein kinase: purification and characterization. J Biol Chem **256:** 5404–5409

Zupan LA, Steffens DL, Berry CA, Landt M, Gross RW (1992) Cloning and expression of a human 14-3-3 protein mediating phospholipolysis. J Biol Chem **267:** 8707–8710