

The Arabidopsis 14-3-3 Family of Signaling Regulators¹

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The 14-3-3 family of proteins has received much attention in the literature during the last 10 years. The current interest is not surprising given the number of diverse organisms in which 14-3-3s have been identified and the important role that they play in signal transduction. Moore and Perez initially catalogued the 14-3-3 proteins in 1967 during an extensive study in which bovine brain proteins were given numerical designations based on column fractionation and electrophoretic mobility (Moore and Perez, 1967). The 14-3-3 family was thought to be limited to nervous tissue and largely conserved among mammals during the late 1960s and 1970s. However, studies over the last 20 years have proven 14-3-3s to be ubiquitous, being found in virtually every eukaryotic organism and tissue (Ichimura et al., 1987; Robinson et al., 1994; Ferl, 1996). In any given organism, the 14-3-3 family usually consists of multiple genes and protein isoforms. Multiple isoforms and multiple functions, coupled with the large number of different organisms that have been studied, have led to potential confusion regarding 14-3-3 nomenclature and function. (14-3-3s are currently designated by Greek letters, with the mammalian isoform names generally chosen from the beginning of the alphabet and the plant isoforms chosen from the end of the alphabet.) The recent completion of the Arabidopsis genome project provides the unique opportunity to examine a complete 14-3-3 family within a single higher eukaryotic organism and to present a framework to codify the understanding of plant 14-3-3 functional diversity and constraint.

The 14-3-3 proteins play key functional roles in many critical physiological pathways that are regulated by phosphorylation. Their role is to complete the signal transduction process by binding to the phosphorylated target, which completes a change in structure that regulates activity. This core functional characteristic is deeply engrained in the highly conserved structural core of the 14-3-3 dimer, which provides grooves for binding two specifically phosphorylated peptides. The primary diversity among 14-3-3 isoforms lies in the N and C termini, with the

C-terminal region potentially able to form a flexible hinge guarding access to the central core region (Sehnke and Ferl, 2000).

Plants require a battery of regulators and corresponding responses to deal with complex environmental and developmental changes, a situation that seems consistent with the presence of a large and diverse 14-3-3 family. Localization of 14-3-3 family members inside organelles such as the chloroplast (Sehnke et al., 2000), nucleus (Bihn et al., 1997), and mitochondria (Sehnke and Ferl, 2000), in addition to the cytoplasm (Bihn et al., 1997), further demonstrates both their global regulatory potential and their apparent need for diversity in expression and function. The list of the processes controlled by 14-3-3s includes the fundamental nitrogen and carbon assimilation pathways, which are executed by the light- and substrate-regulated metabolic enzymes nitrate reductase and Suc phosphate synthase (Sehnke and Ferl, 2000). Other enzymes under the control of 14-3-3s include starch synthase (Sehnke et al., 2001), Glu synthase, F₁ ATP synthase, ascorbate peroxidase, and caffeoyl methyl transferase (Finnie et al., 1999). In addition, the control of the plant's turgor pressure via regulation of at least one form of a plasma membrane H⁺ ATPase is accomplished by 14-3-3 proteins (Korthout and de Boer, 1994; Marra et al., 1994; Oecking et al., 1994). Less understood, yet equally bona fide 14-3-3 binding partners include transcriptional machinery such as the G-box complex and core transcription factors TBP, TFIIB, and EmBP (Chung et al., 1999). The specific 14-3-3 isoforms required by each of these pathways has not been fully characterized; however, a conserved mechanism of plant 14-3-3s binding is the requirement for divalent cations to "charge" the 14-3-3s via a structural reorientation of the C-termini (Lu et al., 1994b). It is interesting that only a subset of the Arabidopsis 14-3-3 isoforms possess this EF hand-like divalent cation-binding motif in the C-terminal region.

The Arabidopsis genome project provides for the first time reasonable certainty about the number and diversity of 14-3-3 family members within a plant species. The Arabidopsis 14-3-3 family consists of 13 members. Ten of the members (omega, phi, chi, psi, upsilon, nu, mu, lambda, kappa, and epsilon) are well characterized and present as expressed sequence tags (ESTs) and cDNAs (Lu et al., 1992; Lu et al., 1994a; Wu et al., 1997). Three of the members (omicron, rho, and pi) are putative members, having

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been identified in GenBank as possessing homology to known Arabidopsis 14-3-3s. The omicron isoform was identified by Rosenquist et al. (2000). A cDNA has not been found for omicron, rho, or pi at the time of this publication. Rosenquist also identified a putative 14th member; however, the isoform is badly truncated and would likely not be functional. Thus, we are designating it as a "14-3-3-like protein" (accession no. AC007264). A table of all the Arabidopsis 14-3-3 proteins and genes with pertinent information is presented in Figure 1.

An alignment of the 13 isoforms reveals some interesting information (see <http://www.hos.ufl.edu/ferllab/> for the alignment). The isoforms range in length from 241 to 268 amino acids. The isoforms all share a conserved core region, with the N and C termini being the most divergent. In fact, the amino acids in the N-termini are conserved to a degree of only 14% and there is very little amino acid conservation at the C-termini (Chung et al., 1999).

Phylogenetic analyses based on amino acid sequence data and gene structure provides a robust tree upon which to hang descriptions of family member function and localization (Fig. 2). The family members break into two major evolutionary branches, the Epsilon group and the Non-Epsilon group. This clear delineation at the trunk of the tree is ubiquitous among plant and animals possessing multiple isoforms, indicating that the initial formation of two isoforms is a fundamental and ancient divergence. The Epsilon group is itself split into the

isoforms epsilon, mu, omicron, rho, and pi. The Non-Epsilon group is made up of the isoforms kappa, lambda, phi, chi, omega, psi, nu, and upsilon. The Epsilon group breaks into two subbranches, with epsilon and pi on one subbranch and omicron, rho, and mu in the second subbranch. The Non-Epsilon group breaks down into three very distinct subbranches. Kappa and lambda make up one subbranch; phi, chi, and omega make up a second subbranch; and psi, nu, and upsilon make up the third subbranch. The Non-Epsilon group members contain the previously mentioned EF hand-like divalent cation-binding motif (Lu et al., 1994b).

The Non-Epsilon and Epsilon groupings are also well supported by intron-exon structure. The Non-Epsilon members all contain four exons and three introns that are highly conserved in placement. Psi, nu, and upsilon contain an extra intron in the 5' leader (Wu et al., 1997). The Epsilon members all possess an intron-exon structure distinct from the Non-Epsilon group, having two additional N-terminal introns. (At present, the pi isoform contains five exons and four introns. The first N-terminal exon is not annotated in GenBank; however, it is present upon inspection.) The genes of the Epsilon group also appear to have additional C-terminal introns. However, the extreme divergence of the C-terminal regions prohibits intron identification based solely on sequence data. Because the omicron, rho, and pi isoforms are not present as cDNAs or ESTs, their structure remains putative at this point.

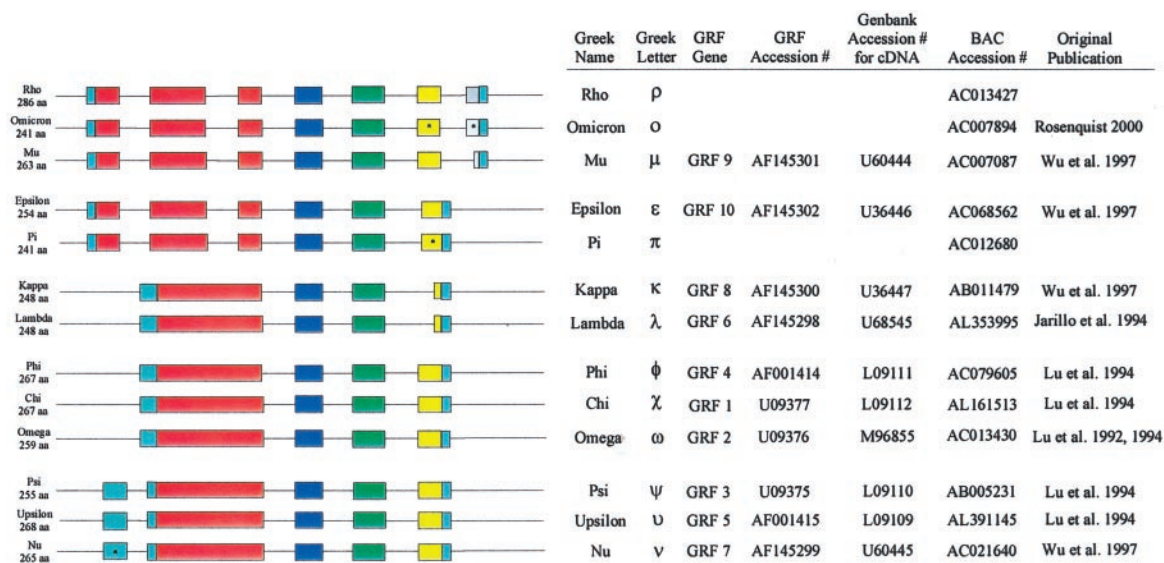


Figure 1. Arabidopsis 14-3-3 gene structures and reference information. Colored boxes represent exons, which are drawn to scale and color coded as to their similarity between genes. The thin lines represent introns, which are not to scale and are shown to denote position only. The light-blue boxes represent 5' leader and 3' trailer sequences. The asterisks identify putative exons, cases where cDNA sequences are not yet available. Current information is outlined to the right of each 14-3-3 gene. Rho, omicron, and pi are the least well characterized of the Arabidopsis 14-3-3s; thus, very little information is available. However, during the review of this manuscript, a cDNA was identified for the Omicron isoform (accession no. AF323920) and a truncated cDNA was identified for the Rho isoform (accession no. AF335544). An expanded version of this table is available at <http://www.hos.ufl.edu/ferllab/>.

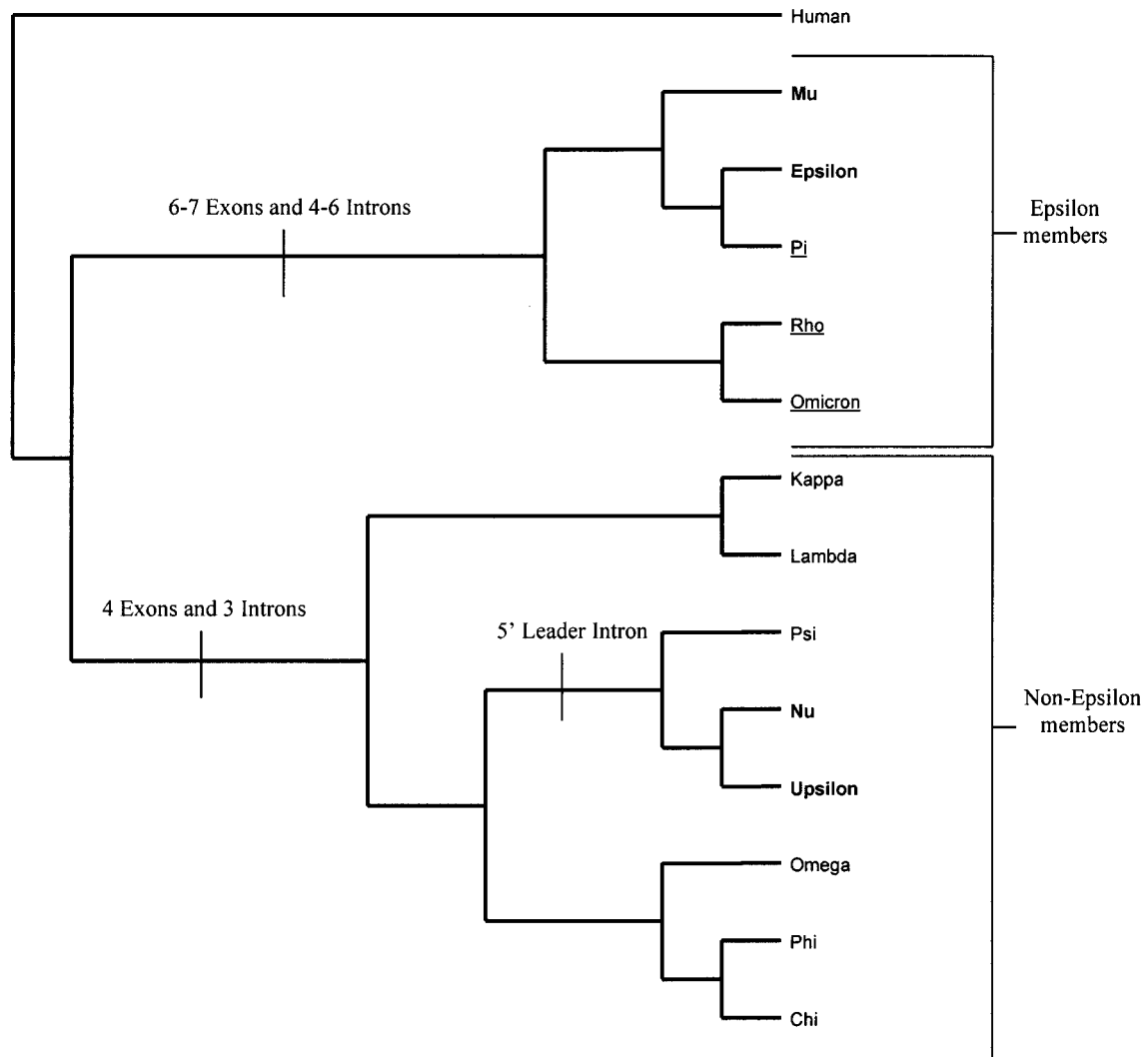


Figure 2. Rooted tree of the Arabidopsis 14-3-3 family. This rectangular cladogram summarizes the relationships among the 14-3-3 isoforms. A human 14-3-3 was included as an outgroup, and essentially roots the tree between the Epsilon and Non-Epsilon groupings. The basic branch topology presented in this neighbor-joining tree is essentially supported by bootstrapped parsimony analysis; however, the confidence of the branching patterns within the Epsilon group is less well supported than the branching within the highly supported Non-Epsilon group. The major branches of the tree are also clearly supported by the gene structures, which is reflected by the numbers of exons and introns as indicated on the branches. Bolded isoforms are present in the chloroplast as well as the cytoplasm. Underlined isoforms are putative in that, as yet, they have not been isolated as cDNAs or ESTs.

The complexity of this phylogenetic tree raises an important question. Why are so many 14-3-3 genes present within a single organism? One possible answer is that there is a need to ensure 14-3-3 activity is present in every compartment of every cell of the organism, suggesting that diversity is simply a reflection of developmental evolution and sophistication. Using current prediction programs, there are no obvious subcellular targeting signals associated with any of the isoforms. Therefore, the large number of isoforms is not obviously linked to diversifying subcellular location. It has been observed, however, that unicellular organisms contain relatively few isoforms, whereas multicellular organisms have many

and certain organelles contain only subsets of the isoforms (Rosenquist et al., 2000). Another possible answer is that each isoform plays a specific and essential biochemical role, suggesting that general diversity reflects functional divergence. They all share a relatively conserved core region, which could point to the conservation of a general theme, yet subtle changes in the core and the divergent termini could give each isoform its specific function by dictating affinity over a range of possible targets.

The structure of this tree does provide an evolutionary perspective that should contribute to answers to these questions based on emerging data. For example, only epsilon, mu, nu, and upsilon are present

in chloroplast stroma, in addition to the cytoplasm (Sehnke et al., 2000), demonstrating that subcellular localization could be consistent with their position on the phylogenetic tree. Omega, chi, and upsilon demonstrate decreasing affinity for nitrate reductase, while phi and psi show no affinity (Bachmann et al., 1996). Isoforms omega, kappa, and lambda demonstrate a decreasing affinity for the proton ATPase (Rosenquist et al., 2000). These examples provide evidence that functional affinity for targets could also be consistent with the phylogenetic tree, but both the localization and function data sets are far from complete. However, the completeness of the Arabidopsis 14-3-3 family should provide a well-developed and inclusive framework for comparative 14-3-3 biology.

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