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Structures and functions of annexins in plants

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Abstract. The first evidence that higher plants contain annexins was presented in 1989. Since that time, annexins have been purified and characterized from a variety of plant sources. Analyses of the deduced proteins encoded by annexin cDNAs indicate that the majority of these plant annexins possess the characteristic four repeats of 70 to 75 amino acids and possess motifs proposed to be involved in Ca^{2+} binding. Like animal annexins, plant annexins bind Ca^{2+} and phospholipids and are abundant proteins, but there are indications that the number of distinct plant annexin genes may be considerably fewer than that found in animals. Regarding function, a number of studies show that various members of the annexin family of plants may play roles in secretion and/or fruit ripening, show interaction with the enzyme callose (1,3-β-glucan) synthase, possess intrinsic nucleotide phosphodiesterase activity, bind to F-actin, and/or have peroxidase activity.

Key words. Annexin; *Zea*; *Gossypium*; *Capsicum*; *Lycopersicon*; actin; peroxidase; glucan.

Discovery of annexins in higher plants

The first evidence that higher plants contain annexin-like proteins was presented in 1989 by Boustead et al. [1]. This work was a collaborative effort between a group of plant scientists and another group working on animal annexins at the University of Leeds. They initiated a search in tomato for proteins that could be precipitated in the presence of Ca^{2+} and phospholipid and that would also crossreact with antibodies against animal annexins. The search proved surprisingly easy since, in retrospect, we now know that plant annexins are apparently both ubiquitous and abundant in plants, usually representing at least 0.1% of the total protein in those cases studied. In the studies on tomato, two proteins of molecular weight 34 and 35 kDa were identified, shown to be precipitated specifically in the presence of Ca^{2+} and phosphatidylserine, and crossreacted with antibodies against two different human annexins. Furthermore, partial peptide sequences were obtained that showed substantial sequence homology with known members of the animal annexin family [1, 2]. Shortly thereafter, another directed search in maize (corn) and lily pollen tubes resulted in isolation by similar procedures of polypeptides of 33 and 35 kDa. A number of peptides from the corn annexins were sequenced and also showed similarity to sequences found in animal annexins [3, 4]. More recently, similar isolations and peptide sequences have been obtained for annexin-like proteins from pea [5], cotton fibres [6], celery [7], pepper [8], and from the rhizoids of ferns [9]. With only a few exceptions, the annexins found were in the range of 33 to 35 kDa. One exception is celery where an annexin-like protein of 42 kDa was identified as being associated in a Ca^{2+}-dependent manner with the vacuolar membrane [7]. To our knowledge, this is the only case of such a localization for an annexin, and it remains to be seen how this particular protein differs in size (e.g. an extended N-terminal region?) and in possible function from other annexins. The second exception is the fern annexin, where the size was shown to be about 70 kDa, which suggests that this annexin might more closely resemble the annexin VI of animals with eight instead of four repeats (see [10, 11] and other articles in this issue for recent reviews of animal annexins); however, the authors did not rule out the possibility that the protein was a dimer of 35 kDa polypeptides that survived SDS-PAGE separation [9]. In sum, it seems that annexins have been found in abundance in every plant where a search was initiated, and that they resemble animal annexins in size and ability to be precipitated in the presence of Ca^{2+} and acidic phospholipids. In at least one case [8], the pepper annexin was also shown to inhibit phospholipase A_2, as found for many animal annexins. Furthermore, the plant annexins also clearly share at least some antigenic determinants and homology at the level of primary amino acid sequence (see [12] for the only other review available on plant annexins).

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Analysis of plant annexin genes and encoded proteins

The fascinating question of the evolution and origin of the repeats within the annexin family has been a topic of some recent interest (e.g. see [13] and Morgan and Fernández, this issue). Recent successes in identification of annexin genes from plants should now begin to add

considerably to our understanding of the evolution of these genes, as well as to enhance further our knowledge of conserved regions within the encoded proteins that may be critical for function. Within the past few years, partial or complete cDNA clones for annexins have now been isolated from alfalfa [14], soybean [15], strawberry [16], pepper [17], cotton [18], corn (Genbank listings X98244 and X98245) and the small crucifer *Arabidopsis thaliana* that has become the model genetic system of higher plants [19]. Of the reported clones, the two genes from cotton were selected by a specific search for annexins that used a polyclonal antibody prepared against the cotton annexins to screen an expression library prepared from mRNA of cotton fibres. The alfalfa clone was isolated more or less by accident while searching for a protein phosphatase gene, while the soybean gene came from a study of cDNA clones that encode proteins associated with plasma membrane of plants. Both the strawberry and pepper cDNA clones came from studies on genes differentially expressed during fruit ripening, while the *A. thaliana* cDNA clone was isolated by its ability to complement the *ΔoxyR* gene of *E. coli*, to be discussed in more detail later. Regardless of the approach used to isolate these cDNA clones, the sequences found show a great deal of similarity to each other, and also show interesting similarities to and differences from their animal counterparts.

Figure 1 shows a phylogenetic tree of annexin proteins derived from the deduced amino acid sequences of major plant cDNA clones compared with a few animal annexins and other annexins lower on the evolutionary scale. The plant annexins definitely represent a unique subset within the overall annexin family, and it would seem that the divergence of the plant-type of annexins from some ancient ancestor, that probably already contained four repeats, occurred rather early in evolution.

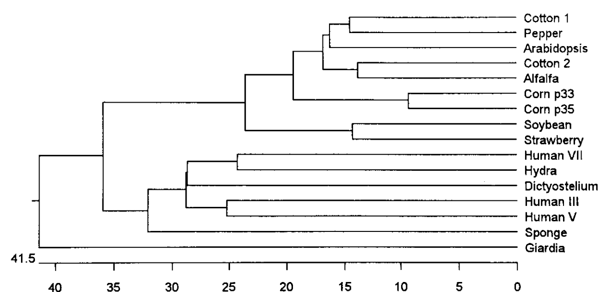

Figure 1. Phylogenetic tree of annexin proteins. The amino acid sequences of the annexins shown were deduced from cDNA clones of the following Genbank accession numbers: cotton 1 (*AnnGh1*): U73746; pepper: (X93308); Arabidopsis: (U28415); cotton 2 (*AnnGh2*): (U73747); alfalfa (*AnnMs*): (X74947); corn p33: (X98244); corn p35: (X98245); soybean: (T41436); strawberry: (U19941); human VII: (A544467); human V: (P08758); human III: (H20560); Hydra: A42660(); Dictyostelium: (S14723); sponge: (S13044); Giardia: (L27221). The tree was prepared using the Lasergene Megalign program (DNA Star, Madison WI); branch distances shown correspond to sequence divergence.

There is some reason now to suspect that the number of distinct annexin gene subfamilies in plants may not be nearly as extensive as that found in animals. For example, from our perusal of the databank of ESTs (expressed sequence tags; randomly-sequenced cDNA clones) from *A. thaliana* that now theoretically covers more than a quarter of the expressed genes of this plant, we found 18 annexin ESTs, 17 of which appear to be derived from the same gene (cloned by Gidrol et al.) [19], with only one other appearing to be a distinct gene. Although the genome size of Arabidopsis is quite small relative to other plants, it has some surprisingly large gene families, such as those for actin and tubulin [20], and the plasma membrane proton ATPase [21], but it appears that the annexin family will not be added to this list. Similarly, genomic Southern blotting with Arabidopsis [19] detected only one major gene, and similar studies with pepper [17] suggested that the gene family may be comprised of only a few members. The phylogenetic tree does hint at some distinctions and possible sub-groupings among the plant annexins; for example, the two cotton genes are not most similar to each other, but rather fall into separate subgroups suggestive of unique functions for them. In any case, the search for plant annexin genes is just beginning, so it is premature to speculate too much about their diversity. Until these genes can be assigned distinct functions, we suggest that the plant annexin community continues to adopt the nomenclature used by Pirck et al. [14] and Potikha and Delmer [18] in which the general abbreviation *Ann* is followed by the initials of the genus and species followed by the gene number in order of date of isolation (e.g. the first annexin gene of cotton, *Gossypium hirsutum*, is called *AnnGh1*). Once functions begin to emerge, a more definite gene terminology may be adopted.

All the plant annexin proteins deduced from the cDNA sequences show the classic four repeats characteristic of these proteins [22]. As an example, figure 2 shows an alignment of these repeats for the cotton AnnGh2 protein compared with the repeats of human annexin V. An analysis of the cotton repeats reveals a situation similar to other annexins, in which repeats 2 and 4 are most similar to each other and repeat 3 is most distinct from the others. Some of the evolutionary studies have also pointed out the striking conservation of introns amongst annexin genes (see [13]; unfortunately, no genomic clones of plant annexins have yet been analysed.

Figure 3 shows a multiple alignment of the plant annexin proteins deduced from the cDNA clones and also includes human annexin V for comparison. Analyses of the alignments shown in figures 2 and 3 reveal the following interesting points: 1) The size, but not the overall sequence, of the plant N-terminal tails is similar (one possible unique function of this N-terminal region is discussed later). 2) Examination of the G × GT loop and the conserved acidic D or E residue found 42 residues downstream, all of which are believed to be

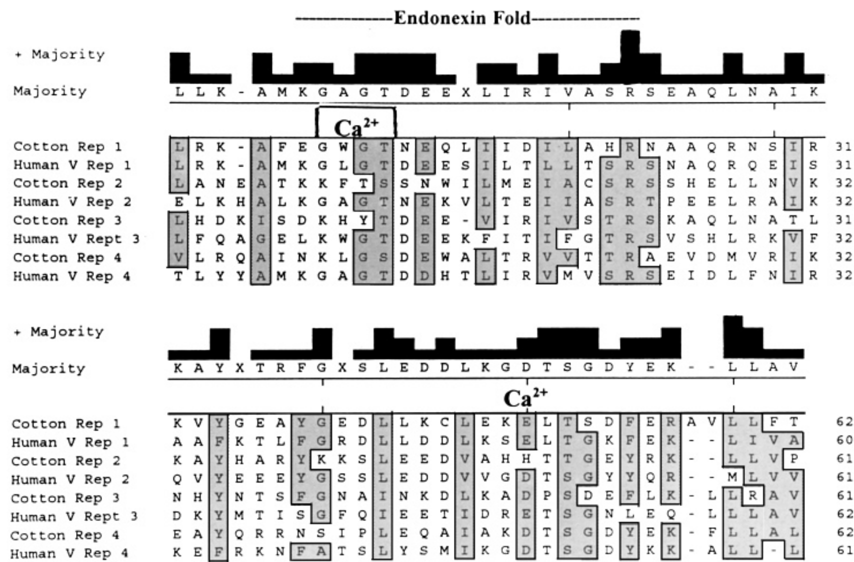

Figure 2. A comparison of the four repeats found in human annexin V with the annexin from cotton (*AnnGh2*). Multiple alignments were performed using the Megalign program described in Figure 1 with gap and gap-length penalties of 10 and PAM 250 weight table. Residues are boxed and shaded when 6 or more match the calculated majority consensus sequence shown above the aligments. The histogram above shows a calculation of the consensus strength for each residue.

involved in binding of Ca^{2+} [23], shows some interesting similarities to and differences from other annexins. In repeat 1 of the plant annexins these residues are highly conserved, and therefore, one can predict that this repeat does bind Ca^{2+}. A similar conservation is found for these residues in repeat 4 of the corn annexins suggesting another Ca^{2+}-binding site in this repeat of these proteins; however, the other plant annexins have an R or K residue substituted for the first G in the motif of repeat 4. In general a similar substitution of the basic R or K for the first G is also found in repeats 2 and 3 of the plant annexins. A similar substitution is found in some of the repeats of some of the non-plant annexins, and, depending upon surrounding residues, may or may not be favourable for binding of Ca^{2+} [23]. One notably distinct feature of the plant annexins is the substitution of an H residue for the conserved acidic residue in repeat 2, a feature that deserves further study in terms of its possible effect on capacity for binding of Ca^{2+}. 3) In addition to the N-terminal region, the greatest divergence amongst the plant annexins is found in the connector region between repeats 2 and 3 and within repeat 3 itself. Therefore, one suspects that if diversity of function can be proved, some of the specificity may lie either in the N-terminal and/or these regions. Furthermore, the potential Ca^{2+}-binding loop in repeat 3 is so divergent that one suspects it may not be involved in cation binding. 4) For the benefit of those interested in the evolution of annexins, we have marked with an asterisk in figure 3 those residues found highly conserved in multiple alignments with annexins from all the diverse genera reported to date.

The overall amino acid sequence similarity among the plant annexins is of the order of 50 to 65% and among animal annexins it is 30 to 40%. Nevertheless, one suspects that there will prove to be a great deal more homology in terms of overall protein structure. A crude example of this is shown in figure 4 where a Kyte-Doolittle hydropathy plot [39] of the cotton AnnGh2 protein shows striking similarity to that of human annexin V.

Gene expression

Little is yet known concerning the pattern of expression of specific annexin genes in plants. Our use of a polyclonal antibody that recognizes both AnnGh1 and AnnGh2 proteins indicated high annexin expression in all major tissues of cotton (Delmer, unpublished). Other immunological studies similarly show expression in most plant tissues, with some preference for higher expression in cells engaged in active secretion or fruit ripening [4, 5, 12, 17, 24]. Similarly, probing with DNA sequences that probably are not gene-specific showed high expression of annexin genes in many plant tissues [14, 25]. These studies point out the need for an array of monoclonal antibodies and gene-specific probes that recognize specific annexins and their genes in plants. However, two cases of enhanced tissue specific expression have been reported for annexins from strawberry [16] and pepper [17] where mRNA levels rose markedly during the process of fruit ripening. As discussed by Wilkinson et al. [16], the discovery of ripening-related annexins is particularly interesting because of the marked changes in membrane properties and cell wall

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Cotton 1      - A T L T - - V F T T V P S V S E D C E - Q - L R K A F S G W G T N E G L I I D I L G H R N A E Q R   45
Arabidopsis   M A T L K - - V S D S V P A P S D D A E - Q - L R T A F E G W G T N E D L I I S I L A H R S A E Q R   46
Pepper        M A S L T - - V F A H V P S A A E D C E - Q - L R S A F K G W G T N E K L I I S I L A H R T A A Q R   46
Cotton 2      - - T L K - - V P V H V P S P S E D A E W Q - L R K A F E G W G T N E Q L I I D I L A H R N A A Q R   45
Alfalfa       - - - - - - - - - S H V P S P S E D S E - Q - L R G A F Q G W G T N E G L I I S I L A H R N A A Q R   39
Corn p33      M A T L K - - V P A T V P P V A D D C D - Q - L R K A F Q G W G T N E A L I I S I L G H R D A A Q R   46
Corn p35      M A T L T - - V P S S V P A V A E D C E - Q - L H K A F E G W G T N E K L I I S I L A H R N A A Q A   46
Soybean       M A T L I - - A P S N - H S P Q E D A E - A - L R K A C X G W G T D E N T V I V I L G H R T V Y Q R   45
Strawberry    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - E R    2
Human V       M A Q V L R G T V T D F P G F D E R A D A E T L R K A M K G L G T D E E S I L T L L T S R S N A Q R   50

Cotton 1      N L I R K T Y A E T - Y G E D L L K A L D K E L S N D F E R L V L L W A L D P A E R D A L V A N E A   94
Arabidopsis   K V I R Q A Y H E T - Y G K D L L K T L D K E L S N D F E R A I L L W T L E P G E R D A L L A N E A   95
Pepper        K L I R Q T Y A E T - F G E D L L K E L D R E L T H D F E K L V L V W T L D P S E R D A H L A K E A   95
Cotton 2      N S I R K V Y G E A - Y G E D L L K C L E K E L T S D F E R A V L L F T L D P A E R D A H L A N E A   94
Alfalfa       K S I R E T Y T Q T - H G E D L L K D L D K E L S S D F E K A V L L W T L D P A E R D A F L A N Q A   88
Corn p33      R A I R R A Y A E A - Y G E E L L R S I T D E I S G D F E R A V I L W T L D P A E R D A V L A N E A   95
Corn p35      R A I R R G Y A E A - Y G K E L L R A L G D E I H G K F E R A V I L W T L D P A E R D A V L A N E E   95
Soybean       Q Q I R R V Y E E I - Y Q E D L V K R L E S E I K G D F E K A V Y R W I L E P A D R D A V L A N V A   94
Strawberry    K E I R A A Y E Q L - Y Q E D L L K P L E S E L S G D F E K A V Y R W T L D P A D R D A V L A N V A   51
Human V       Q E I S A A F K T L - F G R D L L D D L K S E L T G K F E K L I V A L M K P S R L Y D A Y E L K H A   99

Cotton 1      T K R W T S S N Q V L M E I A C T R S A N Q L L H A R Q A Y H A R Y K K S L E E D V A H H T T G D F  144
Arabidopsis   T K R W T S S N Q V L M E V A C T R T S T Q L L H A R Q A Y H A R Y K K S L E E D V A H H T T G D F  145
Pepper        T K R W T K S N F V L V E L A C T R S P K E L V L A R E A Y H A R Y K K S L E E D V A Y H T T G D H  145
Cotton 2      T K K F T S S N W I L M E I A C S R S S H E L L N V K K A Y H A R Y K K S L E E D V A H H T T G E Y  144
Alfalfa       T K M L T S N N S I I V E I A S T R S P L E L L K A K Q A Y Q V R F K K S L E E D V A Y H T S G D I  138
Corn p33      A R K W K P G N R V L V E I A C T R T S A Q I F A T R Q A Y H E R F K R S L E E D I A A H V T G D F  145
Corn p35      A K K S H P G G R A L V E I A C A R T P A Q L F A V K Q A Y H D R F K R S L E E D V A A H V T G D F  145
Soybean       I K S - G K N Y N V I V E I A T I L S P E E L L A V R R A Y L N R Y K H S L E E D V A A H T S G H L  143
Strawberry    I K K S T D V Y N V I I E I S C I H S P E E L L A V R R A Y Q L R Y K H S V E E D L A A H T T G D I  101
Human V       L K G A G T N E K V L T E I I A S R T P E E L R A I K Q V Y E E E Y G S S L E D D V V G D T S G Y Y  149

Cotton 1      H K L L L P L V S S Y R Y E G E E G E H D S G K N R G E V A S E K I S N K A Y S D D D V I R V L A -  194
Arabidopsis   R K L L V S L V T S Y R Y E G D E V N M T L A K Q E A K L V H E K I K D K H Y N D E D V I R I L S -  195
Pepper        R K L L V P L V S S Y R Y G G E E V D L R L A K A E S K I L H E K I S D K A Y S D D E V I R I L A -  195
Cotton 2      R K L L V P L V S A F R Y E G E E V N M T L A K S E A K I L H D K I S D K H Y T D E E V I R I V S -  194
Alfalfa       R K L L V P L V G I H R Y E G D E V N M T L A K S E A K L L H E K I A D K A Y N H D D L I R I V T -  188
Corn p33      R K L L V P L V S T Y R Y D G P E V N T R L A H S E A K L L H E K I H H K A Y S D D E I I R I L T -  195
Corn p35      R K L L V P L V S A Y R Y D G P E V N T S L A H S E A K I L H E K I H K K A Y S D E E I I R I L T -  195
Soybean       R Q L L V G L V T A F R H V G D E I N P                                                            163
Strawberry    R K L L V A L V T A Y R Y D G H E I N A K L A N S E A D I L H D A I K D K A F N H E E I I R I L S -  151
Human V       Q R M L V V L L Q A N R D P D A G I D E A Q V E Q D A Q A L F Q A G E L K W G T D E E K F I T I F G  199

Cotton 1      T R S K A Q I N A T L N H Y K N E Y G N D I N K D L K A D P K D E F L - A L L R S T V K C L V Y P E  242
Arabidopsis   T R S K A Q I N A T F N R Y Q D D H G E E I L K S L E E G D D D D K L P C T L R S T I Q C L T R P E  244
Pepper        T R S K A Q L N A T L N H Y K D E H G E D I L K Q L E D G D E F V A L - - - L R A T I K G L V Y P E  241
Cotton 2      T R S K A Q L N A T L N H Y N T S F G N A I N K D L K A D P S D E F L - K L L R A V I K C L T T P E  242
Alfalfa       T R S K A Q L N A T L N H Y N N E F G N V I D K D L E T D S D D E Y L - K L L R A A I K G L T Y P E  236
Corn p33      T R S K P Q L I A T F N H Y N D A F G H R I N K D L K A D P Q D E Y L - R T L R A I I R C F S C P D  243
Corn p35      T R S K A Q L L A T F N S Y K D Q F T H A I N K D L K A D P K D E F L - S T L R A I I R C F T C P D  243
Soybean                                                                                                          163
Strawberry    T R S K T Q L M A T F N K Y R D D Q G I S I S K N L L E E G A N D F Q K A L H T A I R C L N D - P K  199
Human V       T R S V S H L R K V F D K Y M T I S G F Q I E E T I D R E T S G N L E Q L L L A V V K S I R S I P -  249

Cotton 1      K Y F E K V L R L A I N R R G T D E G A L T R V V C T R A E V D L K I I A D E Y Q R R N S V P L T R  292
Arabidopsis   L Y F V D V L R S A I N K T G T D E G A L T R I V T T R A E I D L K V I G E E Y Q R R N S I P L E K  294
Pepper        H Y F V E V L R D A I N R R G T E E D H L T R V I A T R A E V D L K I I A D E Y Q K R D S I P L G R  291
Cotton 2      Q Y F E K V L R Q A I N K L G S D E W A L T R V V T T R A E V D M V R I K E A Y Q R R N S I P L E Q  292
Alfalfa       K Y F E E L L R L A I N K M G T D E N A L T R V V T T R A E V D L Q R I A E E Y Q R R N S V P L D R  286
Corn p33      R Y F E K V A R Q A I A G L G T D E N S L T R V I T T R A E V D L K L I K E A Y Q K R N S V R L E R  293
Corn p35      R Y F E K V I R L A L G G M G T D E D D L T R V V T T R A E V D L K L I K E A Y Q K R N S V P L E R  293
Soybean                                                                                                          163
Strawberry    K Y F E K V L R N A I K R V G T D E D A L T R V I V T R A E R D L R D I K E V Y Y K K N S V P L E Q  249
Human V       A Y L A E T L Y Y A M K G A G T D D H T L I R V M V S R S E I D L F N I R K E F R K N F A T S L Y S  298

Cotton 1      A I V K D T H G D Y E K L L L V L A G H V E N    315
Arabidopsis   A I T K D T R G D Y E K M L V A L L G E D D A    317
Pepper        A I A K D T R G D Y E S M L L A L L G Q E E D    314
Cotton 2      A I A K D T S G D Y E K F L L A L I G A G D A    315
Alfalfa       A I D K D T S G D Y Q K I L L A L M G H D E      308
Corn p33      A V A G D T S G D Y E S M L L A L L G Q E        314
Corn p35      A V A K D T T R D Y E D I M L A L L G A E        314
Soybean                                                    163
Strawberry    A V A K D T S G D Y K A F L L T L L G K E D      271
Human V       M I K G D T S G D Y K K A L L - L L C G E D D    320
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Figure 3. Multiple alignment of plant and human V annexin protein sequences. The alignment was performed using the Lasergene Megalign program described in figure 1 with gap and gap-length penalties of 10 and PAM 250 weight table. Residues are boxed and shaded when 8 or more of the residues match the calculated majority consensus shown above the sequences. Residues potentially involved in binding of Ca^{2+} are indicated; residues marked with an asterisk were shown to be highly-conserved in another multiple alignment (not shown) that also included other human annexins as well as annexins from other non-plant sources.

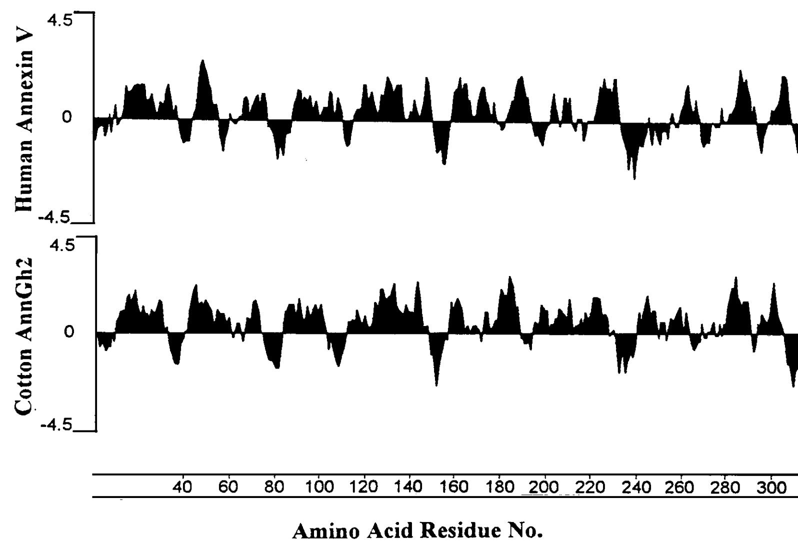

Figure 4. Kyte-Doolittle [39] hydropathy plots comparing human annexin V with cotton annexin AnnGh2. The profiles were performed using the Lasergene Protean program with a window of 7.

structure (which are influenced significantly by Ca^{2+}) that accompany this developmental process. However, further work will be needed to determine the specific role of annexins in this process.

Possible functions of plant annexins

Secretion

Most of the early studies on plant annexins focused upon the possibility that they may be involved in secretory processes, as has been proposed for some animal annexins ([10, 26] and Moss, this issue). However, to date, the evidence for this in plants remains circumstantial. Certainly, the plant annexins interact with phospholipids in a Ca^{2+}-dependent manner (reviewed well in [12, 27, 28]), with the affinity for Ca^{2+} varying from 60 to several hundred micromolar depending upon the system studied. At least in the case of corn, the annexins were also shown to induce aggregation of liposomes or plant secretory vesicles [28]. Immunolocalization studies with pea and corn seedlings showed that annexins were highly concentrated in secretory cell types such as the outer cells of root caps, developing xylem and phloem and epidermal cells [5, 24]. Furthermore, high levels of annexins are found at the tip of pollen tubes of lily [4]. Since pollen tubes are among the few plant cells that elongate by tip growth, this is a cell type that is extremely active in vesicle secretion and fusion with the plasma membrane at the growing tip. Similar results were shown with annexin immunolocalization at the tips of polarly growing fern rhizoids [9].

Possible enzyme activities and/or interaction with other cellular proteins

Callose synthase. Annexins were discovered fortuitously in our laboratory during studies on glucan synthesis in developing cotton fibres [6]. One of the glucan synthases we study is callose synthase (UDP-glc: 1,3-β-glucan synthase). In plants, this enzyme, localized in the plasma membrane, is normally latent and becomes specifically activated upon elevation of intracellular Ca^{2+} (reviewed recently by Delmer and Amor [29]). Thus, callose is often found deposited in response to mechanical damage, environmental stresses, or during pathogenic attack, all conditions in which intracellular Ca^{2+} levels rise. There is no evidence that this activation involves phosphorylation of the enzyme, but more probably results from a direct binding of the cation to the enzyme that lowers the Km for the substrate UDP-glc [30], although this conclusion must remain tentative until the enzyme is purified to homogeneity. We observed that when plasma membranes were prepared in the presence of EGTA, callose synthase activity was quite high, but when prepared in the presence of micromolar levels of Ca^{2+}, callose synthase activity in the plasma membrane was low. Washing of the membranes with EGTA restored high enzyme activity and resulted in the release of a characteristic set of proteins, the most abundant of which proved to be a set of annexins of about 34 kDa that could be resolved into at least three different species by 2D gel electrophoresis. From these proteins in the EGTA wash, the very abundant annexins could be precipitated by Ca^{2+} addition and further purified. This more highly-purified annexin fraction was also able to inhibit the callose synthase activity. We do not yet know the specific interactions that occur to cause this inhibition; in some respects, this inhibitory effect of annexin seems illogical. Since the callose synthase is activated by Ca^{2+}, it does not seem reasonable that interaction with annexin induced by this cation should be inhibitory. We note that Shin et al. have also observed an interaction of callose synthase with annexin

in cotton fibres [31]. They have indicated that the annexin may potentially bind UDP-glc and its analogs, an observation also noted by us [6]. Thus, many questions remain to be resolved concerning this potential role of annexins. That the inhibition of callose synthase by annexin never exceeded 50% suggested to us that annexin might, more importantly, be serving as an anchor for other proteins that might interact with the glucan synthase complex. Certainly, we know that the set of proteins eluted from the plasma membrane by EGTA contains other interesting proteins such as protein kinases [6] and at least some sucrose synthase (Delmer, unpublished). Other recent work of ours has shown that a surprising percentage of sucrose synthase (reaction catalysed: suc + UDP $\rightleftarrows$ UDP-glc + fru), previously thought to be only a soluble enzyme, is associated with the plasma membrane of cotton fibres [32]. This work has provided other evidence to suggest that this form of sucrose synthase may associate with callose and cellulose synthases where it serves to channel carbon from sucrose, via UDP-glc, to the glucan synthases. One of our future goals is to see if annexin may somehow be involved in anchoring accessory proteins such as sucrose synthase or cytoskeletal components to these glucan synthase complexes.

Substrates for protein phosphorylation. Animal annexins I and II can be phosphorylated on tyrosine residues, whereas I, II, IV and XII can be phosphorylated on serine residues by protein kinase C ([10] and Rothhut, this issue). Although some tyrosine phosphorylation occurs in plants, it is apparently less extensive than in animals, and phosphorylation on serine and threonine residues is much more commonly observed. In line with this, the plant annexin sequences detected so far lack the critical Y residue at the N-terminus. However, three of the known plant annexin sequences do contain a T-L-K motif in the N-terminal tail (Arabidopsis, *AnnGh2* from cotton, and p33 of corn, see fig. 3) that closely resembles the motif S/T-V-K, recognized in animal annexins by protein kinase C [10]. The fact that only one of the two corn and cotton annexins contains this motif provides a further suggestion of distinct functions for the two annexins of these plants. The only other indication so far that plant annexins may undergo phosphorylation is our observation that the Ca^{2+}-precipitable annexin proteins from cotton fibres can be phosphorylated, in a reaction that requires Mg^{2+} but is inhibited by Ca^{2+}, by a protein kinase found in the EGTA wash of plasma membranes.

Calcium channels. Animal annexins I, V, VI, and VII all exhibit voltage-gated Ca^{2+} channel activity in vitro ([10, 33] and Huber, this issue). No studies have yet been reported that tested any plant annexins for such activity. However, the striking similarity between the hydropathy plots of a plant annexin with human annexin V (fig. 4) provide a hint that such activities are certainly conceivable for at least some of the plant annexins. The current ability to produce recombinant plant annexins should open the way for such studies and also for the possibility of using X-ray crystallography, that has proved so fruitful for understanding annexin structure in animals ([23] and Huber, this issue).

Interaction with actin; nucleotide phosphodiesterase activity. Animal annexins I and II have both been shown to bind to F-actin in a Ca^{2+}-dependent manner (see [34, 35] and references therein). Plant annexins from corn [4], pepper [8] and cotton (Delmer, unpublished) were all tested for possible interaction with actin with negative results. However, Calvert et al. provide convincing evidence that tomato annexins p34 and p35 bind to F-actin but not to G-actin in reactions that required Ca^{2+} in levels of 100–300 μM [36]. These levels seem high, but it may be possible that microdomains of Ca^{2+} become elevated to rather high levels; alternatively, conditions for actin binding in vitro may not reflect those in vivo. In the same study these tomato annexins were also shown to possess a nucleotide phosphodiesterase activity of rather broad specificity. A variety of nucleoside di- and triphosphates were hydrolysed in a reaction that did not require divalent cations and was inhibited by phospholipids. McClung et al. have detected a similar activity for corn annexins [37]. Calvert et al. have pointed out that previous attempts to detect annexin interaction with actin may have suffered from technical limitations [36]. It will now become important to test other annexins for actin binding and nucleotide phosphodiesterase activities under the conditions used by these authors. One possible function of the ATPase activity might be if the annexin served as a motor protein on actin, but at the moment this is only speculation. Although tomato annexins were the first annexins discovered in plants, it is unfortunate that no cDNA clones for these annexins have yet been reported; having such sequences available would be helpful in searching for common motifs that might be involved in actin binding and/or phosphodiesterase activity.

Plant annexins as peroxidases. One of the most surprising findings to date is the recent report by Gidrol et al. that the major annexin of Arabidopsis may function as a peroxidase [19]. This completely unexpected finding resulted from studies in which a cDNA library from *A. thaliana* was used to screen for cDNAs that could rescue the Δ*oxyR* mutant phenotype in *E. coli*. In *E. coli*, this mutation lies in a gene for a protein that is a transcriptional regulator of a set of genes involved in defence against peroxide stress. Among the cDNA clones from *A. thaliana* that could complement this mutation (i.e. allow it to grow in the presence of H_2O_2), the most effective was a cDNA for what appears to be the most highly-expressed annexin gene of *A. thaliana*. In searching for the mechanism by which an annexin might allow the Δ*oxyR* mutant to grow in the presence

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Residue #      1 2               10          16                   27            34
PER-1          P A P F F T L P - Q L K A A N F K N V G L D R P S D L V A L S G A H
PER-2          P A P F F T L P - Q L K D A - F A K V G L D R P S D L V A L S G G H
PER-3          P S P F F N L T - Q L K T A - F A D V G L N R T S D L V A L S G G H
PER-4          P A P S M S L S - Q L I S S - F S A V G L - S T R D M V A L S G A H
PER-5          P A P F F T L P - Q L K D S - F R N V G L N R S S D L V A L S G G H
PER-6          P S P F F T L A - Q L K K A - F A D V G L N R P S D L V A L S G G H
PER-7          P A P F F N L S - G L I S A - F S N K G F - T T K E L V T L S G A H
PER-8          P A P S K S V D V Q K Q K - - F A A K G L N - T Q D L V T L V G G H
Arab. Annexin  P A P S D D A E - Q L R T A - F E - - G W G T N E D L I I S I L A H
Cotton AnnGh1  P A P S D D A E - Q L R T A - F E - - G W G T N E D L I I S I L A H
Cotton AnnGh2  P S P S E D A E W Q L R K A - F E - - G W G T N E - L I I D I L A H
Corn p33       P P V A D D C D - Q L R K A - F Q - - G W G T N E A L I I S I L G H
Corn p35       P A V A E D C E - Q L H K A - F E - - G W G T N E K L I I S I L A H
Pepper         P S A A E D C E - Q L R S A - F K - - G W G T N E K L I I S I L A H
Human I        F N P S S D V A - A L H K A - I M - - V K G V D E A T I I D I L T K
Human II       F D A E R D A L - N I E T A - I K - - T K G V D E V T I V N I L T N
Human III      F S P S V D A E - A I Q K A - I R - - G I G T D E K M L I S I L T E
Human IV       F N A M E D A - Q T L R K A - M K - - G L G T D E D A I I S V L A Y
Human V        F D E R A D A E T - L R K A - M K - - G L G T D E E S I L T L L T S
Human VI       F D P N Q D A E A - L Y T A - M K - - G F G S D K E A I L D I I T S
Human VII      F D A I R D A E I - L R K A - M K - - G F G T D E Q A I V D V V A N
Human VIII     F N P D P D A E T - L Y K A - M K - - G I G T N E Q A I I D V L T K
Human XI       F D P L R D A E V - L R K A - M K - - G F G T D E Q A I I D C L G S
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Figure 5. Multiple alignment of a heme-binding region from 8 plant peroxidases with N-terminal sequences from plant and human annexins. The concept for the relationship between peroxidases and annexin sequences was originally developed by Gidrol et al. [19]. Alignments were carried out as described in figure 3. Residues are boxed and shaded when 12 or more residues match the residues found in peroxidase 1. The peroxidases numbered 1–8 are from: Arabidopsis (Per-1 and Per-3); horseradish (Per-2, Per-3, Per-6); turnip (Per-4); peanut (Per-7, Per-8). Original references for these peroxidases are cited in Gidrol et al. [19].

of high levels of H_2O_2, the authors noted that the N-terminal region of the Arabidopsis annexin shared some sequence homology with a conserved heme-binding region of many plant peroxidases. Indeed, when tested, the recombinant annexin did display peroxidase activity. To our knowledge, this is the first report of an annexin from any organism to possess such activity. In figure 5, we have further extended the comparison made by Gidrol et al. [19] and show a multiple alignment of the N-terminal of several plant annexins deduced from some of the longer cDNA clones available with the heme-binding region of eight different plant peroxidases as well as with the corresponding N-terminal region of human annexins. As pointed out by Gidrol et al., there is a critical conserved histidine residue in all the sequences that have peroxidase activity and are known to be involved in heme binding (marked with an asterisk in fig. 5). Other nearby conserved residues were also noted by them [19]. What emerges from the comparison in figure 5 is that the residues 1 (P), 2 (A/S), 10 (Q), 16 (F), 27 (L) and 34 (H) as shown, are uniquely conserved in the peroxidases and plant annexins, but not in annexins from other sources.

Does this mean all plant annexins are peroxidases? One somehow suspects not, although there is surprising conservation of these sequences in all the plant annexins so far examined. However, it is much too soon to make predictions, since so few N-terminal sequences are available and it will take time to test purified annexins for this activity. One curiosity not noted by Gidrol et al. [19] is that this motif sits within the endonexin fold of repeat 1 (see fig. 2) of these annexins, the most likely of all regions in the protein predicted to bind Ca^{2+}. Thus, it becomes intriguing to speculate whether these annexins might have peroxidase activity when Ca^{2+} levels are low, and whether elevation of Ca^{2+} could lead to heme displacement and inhibition of the activity.

In sum, plants clearly contain annexins with some interesting similarities and differences from those found in other organisms. It is becoming clear that plants share many similarities with animals in terms of regulatory mechanisms relating to Ca^{2+}. For example, plants have calmodulin, an inositol triphosphate pathway, small G-proteins, and protein phosphorylation cascades, some of which are similar to those found in animals (for reviews of regulation by Ca^{2+} in plants, see [21, 38]). Thus, it is perhaps not surprising that plants contain annexins as well. Clearly, the field of plant annexin research is rapidly gaining momentum. Much progress can be predicted in the near future, and it may well be that the emerging findings on these abundant plant proteins will begin to shed further light on the evolution, structure and function of the entire annexin family.

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