Annexins: multifunctional components of growth and adaptation

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Abstract

Plant annexins are ubiquitous, soluble proteins capable of Ca²⁺-dependent and Ca²⁺-independent binding to endomembranes and the plasma membrane. Some members of this multigene family are capable of binding to F-actin, hydrolysing ATP and GTP, acting as peroxidases or cation channels. These multifunctional proteins are distributed throughout the plant and throughout the life cycle. Their expression and intracellular localization are under developmental and environmental control. The in vitro properties of annexins and their known, dynamic distribution patterns suggest that they could be central regulators or effectors of plant growth and stress signalling. Potentially, they could operate in signalling pathways involving cytosolic free calcium and reactive oxygen species.

Key words: Annexin, calcium, channel, GTP, peroxide, stress.

Introduction

Plants sense and respond to a range of environmental, metabolic, and developmental signals. Operation and control of interacting signal transduction pathways will involve cell and endomembranes, and integral, peripheral, and soluble proteins. Downstream responses may require remodelling of the cytoskeleton and changes to exocytotic machinery and walls. One family of plant proteins appears to have the capacity to function at all of those levels—the annexins. What are they? First discovered in plants (tomato) by Boustead et al. (1989), research interest in plant annexins has been spasmodic. In contrast, animal annexins have been studied extensively. Animal annexins are involved in signal transduction, free cytosolic Ca²⁺ ([Ca²⁺]cyt) homeostasis, exo- and endocytosis, membrane organization, cytoskeletal dynamics, cell cycle control, and water permeability (Gerke and Moss, 2002; Hill et al., 2003; Gerke et al., 2005). Analogous functions in plants could place annexins centre stage in signalling and adaptation. They are already implicated in cold, oxidative, saline, and abscisic acid (ABA) stress responses. What do we know about them and could they be important components of signalling and response? Here, plant annexin proteins, their localization, and possible roles are reviewed.

Annexins of the plant kingdom

Annexins are a multigene, multifunctional family of soluble proteins with a broad taxonomic distribution. Over 200 unique annexin sequences have been described in >65 species covering plants, fungi, protists, higher vertebrates, and recently a prokaryote (reviewed by Gerke and Moss, 2002; Hofmann, 2004; Moss and Morgan, 2004; Morgan et al., 2006). Most of what we know comes from studies on mammalian annexins. Little is known about the phylogenetically distinct plant annexins (Fig. 1). They have been found in all monocot and dicot plants tested to date (reviewed by Delmener and Potikha, 1997; Hofmann, 2004), including the model plant Arabidopsis (Clark et al., 2001) and the model legume Medicago (Kovács et al., 1998; de Carvalho-Niebel et al., 1998, 2002).

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Genome sequencing has revealed seven annexin genes in Arabidopsis (with an eighth evident; Cantero et al., 2006) and nine in rice (Moss and Morgan, 2004). Annexins are expressed throughout the body and lifespan of the plant; embryo (Yu et al., 2005), seedlings (Clark et al., 1992, 2001; Proust et al., 1996; Cantero et al., 2006), roots and tubers (Gidrol et al., 1996; Carroll et al., 1998; de Carvalho-Niebel et al., 1998; Kovács et al., 1998; Lim et al., 1998; Clark et al., 2001, 2005a, b; Bassani et al., 2004; Hoshino et al., 2004; Bauw et al., 2006; Cantero et al., 2006), cotyledons and leaves (Kovács et al., 1998; Santoni et al., 1998; Hofmann et al., 2000; Seigneurin-Berny et al., 2000; Hoshino et al., 2004; Cantero et al., 2006), inflorescence (Blackbourn et al., 1991; Thonat et al., 1997; Kovács et al., 1998; Hoshino et al., 2004; Cantero et al., 2006), stems, hypocotyls, and coleoptiles (Blackbourn et al., 1991; Thonat et al., 1997; Kovács et al., 1998), and fruit (Wilkinson et al., 1995; Proust et al., 1996; Hofmann et al., 2000). In addition to expression in the vasculature (Clark et al., 2001), annexin proteins have been found in phloem sap (Barnes et al., 2004; Giavalisco et al., 2006). It is estimated that annexins can comprise 0.1% of plant cell protein (Blackbourn et al., 1991).

Proteomic studies now show that plant and oomycete annexins exist in the cell wall as well as the cytoplasm (Kwon et al., 2005; Meijer et al., 2006). To date, plant studies have focused on annexin structure and in vitro protein function. Such studies have revealed a capacity for annexins to be multifunctional and point towards possible in vivo roles.

**Plant annexin structure differs from that of animal annexins**

Plant annexins have a molecular weight in the region of 32–42 kDa and, although sharing a common evolutionary ancestor, differ structurally from their animal counterparts. Animal annexins consist of a conserved α-helical core and a variable N-terminal region. Their annexin core is constructed from annexin domains (usually found repeated four times within the protein) each comprising a 70 amino acids, seven-stranded a-helical core and a variable N-terminal region. It is thought that this region
regulates the stability of different annexin conformations, determines interactions with other proteins, and hence is responsible for the functional diversity of mammalian annexins (Gerke and Moss, 2002). It is the site of secondary modification, including phosphorylation, nitrosylation, S-glutathiolation, and N-myristoylation, indicative of regulation by several distinct signalling pathways (reviewed by Gerke and Moss, 2002; Gerke et al., 2005).

For plant annexins, typically only the first and fourth repeated domains have the characteristic endonexin sequence (Fig. 2). Crystal structures have been described (Hofmann et al., 2000, 2003). Plant annexins have, on average, a larger surface area than mammalian annexins (Clark et al., 2001). This is due to extra grooves and clefts, perhaps suggesting a wide range of interaction partners and a broad range of roles within the cell. However, in contrast to their animal counterparts, the N-terminal region of all known plant annexins is short (~10 amino acids). The crystal structure of bell pepper annexin (AnxCa32) revealed that the short N-terminal
region interacts with the annexin core, suggesting that some regulatory function of this region is conserved in plant annexins (Hofmann et al., 2000).

**Ca\textsuperscript{2+}-dependent and -independent membrane binding**

Characteristically, both animal and plant annexins bind Ca\textsuperscript{2+} and, in the presence of (micromolar) Ca\textsuperscript{2+}, will bind to negatively charged phospholipids including phosphatidylserine, phosphatidylinositol, and phosphatidic acid (Blackbourn et al., 1991; Balasubramanian et al., 2001). Binding can be reversed by addition of Ca\textsuperscript{2+} chelators. An annexin may be membrane associated or even membrane inserted, depending on the [Ca\textsuperscript{2+}]\textsubscript{cyt}, pH, lipid composition, and voltage (reviewed by Gerke and Moss, 2002). Certain annexins such as AnxB12 from *Hydra* have the capacity to be soluble, peripheral, and integral proteins (Ladokhin and Haigler, 2005). Using molecular simulation and site-directed mutagenesis, Montaville et al. (2002) identified a consensus phosphatidylserine-binding site ([R/K]XXXK-BC-helices-[R/K]XXXXDXXS[D/E]+Ca\textsuperscript{2+}) in mammalian annexins. Found in domain I and sometimes additionally in domain II (but never in domains III or IV), the site overlaps the Ca\textsuperscript{2+}-binding domains. Sequence alignment with mammalian annexins revealed that the phosphatidylserine-binding site is only poorly conserved in plant annexins (Hofmann et al., 2000). Despite divergence in amino acid sequence, phosphatidylserine-binding activity is conserved in at least some plant annexins including maize, bell pepper, and cotton (Blackbourn et al., 1991; Hofmann et al., 2000; Dabitz et al., 2005). Strict sequence conservation does not appear to be necessary for membrane-binding function. This is supported by the observation that both plant and animal annexins bind to a range of negatively charged phospholipids in addition to phosphatidylserine, including phosphatidylinositol, phosphatic acid, and malonaldehyde-conjugated lipids (Blackbourn et al., 1991; Balasubramanian et al., 2001). In plants, hydrophobic interactions are also involved in membrane binding. AnxCa32 attachment to membranes involves the hydrogen bonding of several amino acid residues to the phospholipid headgroup and glycerol backbone (Dabitz et al., 2005). Site-directed mutagenesis of recombinant tomato annexin p35 (AnxLe35) revealed that the fourth repeat of the core domain was critical to lipid binding (Lim et al., 1998).

Although Ca\textsuperscript{2+} is required for membrane binding at neutral pH, at acidic pH (< pH 6.0) some animal annexins bind to membranes independently of Ca\textsuperscript{2+} (Köhler et al., 1997; Langen et al., 1998; Rosengrath et al., 1998; Golczak et al., 2004). Plant annexins also appear capable of Ca\textsuperscript{2+}-independent membrane binding. Recently, it has been reported that ~20% of the annexin protein (AnxGh1 and AnxCa32) remains bound to lipid vesicles in the absence of Ca\textsuperscript{2+} at neutral pH, and the proteins can be released following addition of detergent (Dabitz et al., 2005). However, rather than Ca\textsuperscript{2+}-independent membrane binding, as suggested by Dabitz et al. (2005), this may represent a proportion of the population undergoing membrane insertion, hence the requirement for detergent to release the protein. In addition to promoting Ca\textsuperscript{2+}-independent binding, acidic pH can reduce the Ca\textsuperscript{2+} requirement for phosphatidylserine binding (Blackbourn et al., 2001).
et al., 1991). The mechanism of plant annexin Ca\textsuperscript{2+}-independent membrane binding is still unclear, although a pair of conserved tryptophans (W35/W107) is involved (Dabitz et al., 2005). Additionally, it has been suggested that Ca\textsuperscript{2+}-independent membrane binding serves as a platform for an annexin population whose membrane binding is Ca\textsuperscript{2+} dependent. Given that sequential and co-operative binding of annexins has been shown, the two modes of membrane binding may be intimately linked (Dabitz et al., 2005).

Although the majority of plant annexins tend to be found in the cytosol (Blackbourn et al., 1992; Clark et al., 1992, 1994; Thonat et al., 1997), they are also found bound to (or in some cases inserted into) both plasma membranes and endomembranes (Fig. 3B). Annexins can localize to the plant vacuolar membrane (Seals et al., 1994; Seals and Randall, 1997; Carter et al., 2004), the Golgi, and Golgi-derived vesicles (Clark et al., 1992). Plant annexins can also cause aggregation of liposomes and secretory vesicles, implicating them in membrane organization (Blackbourn and Battey, 1993). Medicago truncatula annexin1 (AnxMt1; de Carvalho-Niebel et al., 1998) localizes to the nuclear membrane, and M. sativa (the model legume) AnxMs2 has been shown to localize in the nucleolus under stress conditions even though the protein shows no typical nuclear localization signal (Kovács et al., 1998). A nuclear localization has also been reported for pea annexin (Clark et al., 1998). A putative spinach annexin has been identified in chloroplast envelope membranes (Seigneurin-Berny et al., 2000). Arabidopsis AnxA1 has been found in chloroplasts (Peltier et al., 2002, 2006; Friso et al., 2004; Kleffmann et al., 2004; Renaut et al., 2006) but also as a tonoplast protein (Carter et al., 2004) and an integral plasma membrane protein ostensibly under non-stressed conditions (Santoni et al., 1998; Alexandersson et al., 2004). Its membrane association is prompted by salinity stress (Lee et al., 2004). Arabidopsis AnxA4 has also been identified as a plasma membrane protein (Alexandersson et al., 2004). An annexin from Bryonia dioica relocates from the cytoplasm to the plasma membrane following mechano-stimulation (Thonat et al., 1997). In wheat exposed to low temperatures, two wheat annexins accumulate in the plasma membrane (Breton et al., 2000). Moreover, they are integral membrane proteins, which cannot be released by addition of Ca\textsuperscript{2+} chelators (Breton et al., 2000). That annexin association with or insertion into membranes can be dynamic and responsive to environmental change is consistent with their involvement in signalling and adaptation.

**Actin binding**

Actin filaments help shape a cell, are essential for the development of certain plant cell types, and act in signalling (reviewed by Drůbak et al., 2004). Actin binding is limited to a small number of animal annexins and, as a generalization, actin–annexin interaction appears to be restricted to regions close to animal membranes (Hayes et al., 2004). The molecular mechanism of actin binding is poorly understood, but a C-terminal motif (LLYLCGGDD) has been implicated; this is not present in plant annexins (reviewed by Hayes et al., 2004; Konopka-Postupolska, 2007). Evidence for binding of plant annexins to actin is mixed, and appears to be species specific. Tomato and mimosa annexins both undergo Ca\textsuperscript{2+}-dependent F-actin binding *in vitro* (Calvert et al., 1996; Hoshino et al., 2004). Two plasma membrane-associated annexins from zucchini bind to zucchini-derived F-actin (Hu et al., 2000). Mimosa annexin organizes F-actin into thick bundles in the presence of 2 mM Ca\textsuperscript{2+} *in vitro* (Hoshino et al., 2004). Cotton, bell pepper, and maize annexins have all been extensively tested and show no affinity for actin, in either the presence or absence of calcium (Blackbourn et al., 1992; Delmer and Potikha, 1997; Hoshino et al., 2004). However, the latter all possess the IRI motif (needed in F-actin binding to myosin) implicated in actin binding by Lim et al. (1998) for tomato annexin. This suggests that the structural requirement for actin binding is more complex. As yet annexins from two of the most studied species, Arabidopsis and Medicago, have not been tested for F-actin binding, although Arabidopsis sequences contain a full or partially conserved F-actin-binding motif (Clark et al., 2001). The functional significance of annexin–actin interaction is unknown, but has been postulated to be involved in exocytosis and signalling (Konopka-Postupolska, 2007).

**Peroxidase activity**

The crystal structure of cotton annexin AnxGh1 revealed two adjacent cysteine residues which, in combination with a nearby methionine residue, form an S3 cluster. Although no function has been demonstrated for this cluster, it has been suggested that it may have a role in transfer of electrons to an oxidizing molecule, potentially a reduced reactive oxygen species (ROS) (Hofmann et al., 2003). Although experimental studies have been limited to a single Arabidopsis annexin, it is possible that several plant annexins may be able to act as peroxidases. Heterologous expression of Arabidopsis annexin AnxA1 in the Escherichia coli oxyR (peroxide-sensitive) mutant or overexpression in mammalian cells afforded protection against peroxide-mediated oxidative stress (Gidrol et al., 1996; Kush and Sabapathy, 2001). Peroxidase activity was demonstrated *in vitro* using both recombinant AnxA1 and AnxA1 purified from Arabidopsis tissue (Gidrol et al., 1996; Gorecka et al., 2005). Post-translational modification of AnxA1 may be required for peroxidase
activity as recombinant AnxA1 purified from *E. coli* had lower activity than that purified from *Nicotiana benthamiana*, the activity of which was decreased by dephosphorylation (Gorecka et al., 2005). Indeed, secondary modification of plant annexins is suggested by SDS–PAGE analysis. For example, the theoretical size and isoelectric point of AnxA1 are 36 kDa and pl 5.2, respectively, whereas following two-dimensional electrophoresis Lee et al. (2004) found two microsomal AnxA1 spots: 40 kDa, pl 5.2; and 40 kDa, pl 5.3. Santoni et al. (1998) reported two plasma membrane forms of AnxA1 with the following migration characteristics: 39 kDa, pl 5.0; and 34 kDa and pl 5.1. Additionally, annexin nitrosylation has been observed in *Arabidopsis* (Lindemeyer et al., 2000).

The peroxidase activity of AnxA1 is suggested to be due to a region of the first annexin domain in the N-terminal region (centring on a conserved histidine residue; His40) which has a strong sequence similarity to the ~30 amino acid haem-binding motif of plant peroxidases, typified by horseradish peroxidase (Clark et al., 2001; Gorecka et al., 2005). Consistent with this idea, mutation of the conserved histidine to alanine substantially reduced in vitro peroxidase activity (Gorecka et al., 2005). The putative haem-binding motif is conserved in several plant annexins including those of maize, cotton, and bell pepper (Fig. 2). Haem-containing peroxidases catalyse the oxidation of a substrate by the removal of a single electron and reduce hydrogen peroxide to water. However, to date there have been no reports of haem binding to plant annexins to confer peroxidase function. Neither are the potential targets of annexin peroxidase activity identified as yet. It remains feasible that in vitro peroxidase activity is a consequence of metal-binding ability generating artificial catalytic sites. It is feasible that in common with other types of peroxidases, annexins are capable of generating ROS. However, the sheer numbers of peroxidases in plants and potential for redundancy may make this aspect of annexin physiology particularly difficult to elucidate.

### ATPase and GTPase activity

Binding to ATP and GTP is a common feature of animal annexins even though they do not contain the Walker A and B motifs. Rather, the nucleotide-binding motif is thought to be FXXKYD/EKSL (Bandorowicz-Pikula et al., 2003). Plant annexins not only bind purine nucleotides but also hydrolyse them (maize, McClung et al., 1994; tomato, Calvert et al., 1996; Lim et al., 1998; cotton, Shin and Brown, 1999). In contrast to animal annexins, nucleotide binding and hydrolysis may depend on a Walker A motif (GXXXXGKT/S) and a GTP-binding motif typical of the GTPase superfamily (DXXG). These sequences have been found in the fourth repeat of AnxGh1; C-terminal deletion and loss of the fourth repeat abolished its GTPase activity (Shin and Brown, 1999). In *Arabidopsis*, the greatest similarity is in the fourth repeat of AnxA2 and AnxA7 (Clark et al., 2001).

Both maize and tomato annexins are able to hydrolyse ATP and GTP at a similar rate, but GTP is the preferred substrate for cotton annexin AnxGh1. Tomato annexin GTPase activity still proceeds when the protein is bound to actin (Calvert et al., 1996), suggesting that cytoskeletal association may specifically locate the annexin’s GTPase function in the cell. Ca²⁺ has an inhibitory effect on cotton annexin GTPase activity but not the ATPase/GTPase activity of maize annexin AnxZm33/35 (McClung et al., 1994; Shin and Brown, 1999). Alignments of the primary sequence of cotton annexin, AnxA1, and AnxZm33/35 showed that the GTP-binding motifs overlap the Ca²⁺-binding motif of the fourth endonexin domain (McClung et al., 1994; Shin and Brown, 1999). Ca²⁺ and GTP may therefore compete for binding. Ca²⁺-mediated phospholipid binding has been shown to inhibit hydrolytic activity of tomato annexin (Calvert et al., 1996). Mutagenesis of the Ca²⁺-binding sites does not impair GTPase activity of the soluble form (Lim et al., 1998), demonstrating that membrane binding prevents GTP from reaching its catalytic site. Overall, modulation of enzyme activity by Ca²⁺ and membrane binding may afford spatio-temporal definition of annexin function. That tomato, maize, and cotton annexins have different requirements, despite catalysing the same reaction, may reflect the diverse roles that plant annexins fulfil.

### Sensor and channel activity

Ca²⁺ binding is a defining annexin characteristic, but studies on animal annexins show them to be capable of sensing and regulating free cytosolic calcium (Hawkins et al., 2000; Watson et al., 2004). Mammalian AnxA6 has been shown to act as a Ca²⁺ sensor, mediating membrane association of a GTPase-activating protein (GAP) that then regulates a monomeric GTPase (Grewal et al., 2005). In addition to controlling trafficking of ion transporters to their target membranes and regulating their activity (reviewed by Gerke et al., 2005), animal annexins can also form Ca²⁺-permeable ion channels themselves. This ability was first demonstrated with bovine AnxA7 which forms a highly selective, voltage-gated Ca²⁺ channel in phosphatidylserine bilayers (Pollard and Rojas, 1988). Since then, many other animal annexins have been shown to form ion channels, although their properties (particularly selectivity) differ (reviewed by Hawkins et al., 2000; Kourie and Wood, 2000). ATP, GTP (Kirilenko et al., 2006), cAMP, hydrogen peroxide (Kubista et al., 1999), and low pH (Köhler et al., 1997; Langen et al., 1998; Rosengarth et al., 1998) have all been shown to regulate annexin channel activity. Animal annexins can cause Ca²⁺
influx across animal plasma membrane and mobilize Ca\(^{2+}\) from internal stores (Kubista et al., 1999; Watson et al., 2004) placing them at the core of Ca\(^{2+}\)-mediated signal transduction and Ca\(^{2+}\) homeostasis.

A number of models have been proposed to explain the mechanism whereby animal annexin proteins (which are too small to span a membrane) form ion channels. At acidic pH, the short helices of the monomer may join to form longer helices to allow spanning of the membrane. In invertebrates this has been observed for AnxB12, which undergoes large-scale conformational changes under conditions that result in membrane insertion (Langen et al., 1998; Isas et al., 2000). Recently, freeze-fracture electron microscopy revealed images of AnxB12 as an integral membrane protein at pH 4 but not at higher pH (Hegde et al., 2006). This membrane-inserted form of AnxB12 appears to be monomeric, unlike the trimeric membrane-attached form (Ladokhin and Haigler, 2005). Another model suggests that an annexin monomer associates with the membrane and causes an electrostatic disruption which allows passage of ions through the central pore of the protein, although it is not clear how this pore would be selective for Ca\(^{2+}\) (reviewed by Kourie and Wood, 2000). Regardless of the mechanism, annexin ion channels challenge the view of ion channels as solely integral membrane proteins. As unusual as this is, membrane insertion of ion channels from the aqueous phase is not unprecedented. In addition to annexin channels, an animal chloride channel (CLIC-1; Tulk et al., 2002) also moves directly from the cytosol into a membrane.

An ability of plant annexins to form or regulate Ca\(^{2+}\) channels in plasma and endomembranes would enable signal transduction and amplification (Kovács et al., 1998; White et al., 2002). The putative pore region of the human AnxA5 channel contains two salt bridges (Asp92–Arg117 and Glu112–Arg271) that regulate selectivity for calcium and channel opening in response to voltage (Liemann et al., 1996). Both salt bridges are quite well conserved in plant annexins. It has been proposed that plant annexins could act as the plasma membrane Ca\(^{2+}\)-permeable channels that mediate Ca\(^{2+}\) entry into the cell at very negative (hyperpolarized) membrane voltage (White et al., 2002). Such channels are strongly implicated in growth and signalling. To date, AnxCa32 has been shown to mediate passive Ca\(^{2+}\) influx in fura-2-loaded vesicles (Hofmann et al., 2000), supporting the general concept of channel function. Maize annexin AnxZm33/35 contain the putative salt bridges, and a partially purified preparation formed hyperpolarization-activated Ca\(^{2+}\)-permeable channels in planar lipid bilayers (Nichols, 2005). As only one gene has been verified as encoding a plant Ca\(^{2+}\) channel (TPC1 encoding a vacuolar channel; Peiter et al., 2005), it will be of great interest to see whether plant annexins purified to homogeneity support Ca\(^{2+}\) channel activity. Of the eight Arabidopsis annexins, AnxA1 has been found to form K\(^{-}\)-permeable channels in bilayers, with channel formation favoured at low pH (Gorecka et al., 2007). In common with AnxA5 and AnxB12, acidic pH increases the hydrophobicity of AnxA1 and promotes the oligomerization thought necessary for transport activity. The Ca\(^{2+}\) permeability of the AnxA1 channel remains to be determined, as does its in vivo function, but AnxA1 illustrates clearly the multifunctional nature of annexins—potentially a peroxidase and a channel in one.

### Function in exocytosis, growth, and development

Annexin expression is dynamic even under normal growth conditions. Transcript levels of annexin genes in Arabidopsis vary depending on tissue type and age, suggesting specific purposes at different developmental stages in different tissue (Clark et al., 2001, 2005a; Hoshino et al., 2004; Cantero et al., 2006). Annexin expression increases during fruit ripening and gall ontogeny, implying hormonal control (Wilkinson et al., 1995; Proust et al., 1996; Vandeputte et al., 2007). Nod factors induce *M. truncatula* annexin1 (AnxA1) expression, and co-localization studies using AnxA1–GFP (green fluorescent protein) have suggested that it may be involved in the early stages of cell division required for nodule formation (de Carvalho-Niebel et al., 2002).

The in vitro ability to bind membranes, Ca\(^{2+}\), purine nucleotides, and actin predicts critical roles for both animal and plant annexins in membrane trafficking and signal transduction. Animal annexins are clearly involved in exo- and endocytosis, and the targeting of proteins to specific membrane sites (reviewed by Gerke and Moss, 2002; Gerke et al., 2005). The clearest demonstration to date of annexin function in planta has been the stimulation of Ca\(^{2+}\)-dependent vesicle fusion to the plasma membrane of maize root cap proplastos by AnxZm33 and 35 (Carroll et al., 1998). Plant annexins are abundant and underlie the plasma membrane in cells associated with high secretion rates. Annexins are prominent at the apex of cells undergoing polar elongation, such as root hairs, pollen tubes, and fern rhizoids (Blackbourn et al., 1992; Blackbourn and Battey, 1993; Clark et al., 1995, 2001, 2005a). Annexin expression has been detected in the root elongation zone of maize (Carroll et al., 1998; Bassani et al., 2004) and Arabidopsis (Clark et al., 2005a, b). As well as possibly being involved in primary and root hair elongation growth, a specific annexin in Arabidopsis (AnxA12) is implicated in lateral root development (Clark et al., 2005a), implying upstream regulation by growth regulators. Cotton annexin (AnxGh1) mRNA is up-regulated during cotton fibre elongation, and the protein may be associated with Golgi-derived coated vesicles mediating fibre elongation (Shin and Brown, 1999; Bulak Arpat et al., 2004).
Recent work on a *Saprolegnia* annexin has revealed an ability to stimulate (1–3)-β-D-glucan synthase activity, implying a role in the regulation of wall synthesis (Bouzenenza et al., 2006). This is in contrast to the inhibitory effect of cotton annexin AnxGh1 (Andrawis et al., 1993) and suggests that different annexins play distinct regulatory roles. *Arabidopsis* AnxA7 expression is up-regulated by oxylipin treatment that induces callose formation and causes wavy roots and lateral root inhibition (Vellosillo et al., 2007). It will be interesting to see whether this annexin regulates glucan synthesis. In addition to roles in exocytosis and wall synthesis, individual annexins could be acting as Ca\(^{2+}\) or GTP sensors to co-ordinate growth. A role as a Ca\(^{2+}\) sensor has been proposed for vacuole-associated annexins (Seals et al., 1994). Vacuolar biogenesis is a key component of cell expansion, and expression of the vacuole-associated tobacco annexin VCaB42 correlates with vacuolar biogenesis in expanding cells (Seals and Randall, 1997). The annexin is associated with a ROP GTPase (Lin et al., 2005), a type of protein viewed as master regulators of growth. Addition of GTP inhibited annexin-mediated exocytosis in root cap protoplast (Carroll et al., 1998), suggesting that annexin function is co-ordinated by local GTP and Ca\(^{2+}\) levels.

**Light response, nyctinastic movement, and gravitropism**

As well as being linked to growth and development, annexin expression and distribution can also change in response to environmental stimuli. Light affects the expression of certain annexins in *Arabidopsis* (Cantero et al., 2006). In hypocotyls, AnxA5 expression is increased by red light and this is reversible by application of far red light; in cotyledons, AnxA6 has a similar red/far red response (Cantero et al., 2006). These results point to annexin expression being downstream of phytochrome A, and further dissection of this relationship is awaited. Phytochrome action has previously been implicated in the regulation of polarized annexin distribution in fern rhizoids (Clark et al., 1995). Nyctinastic (night time) movement of the *Mimosa* pulvinus provides a beautiful example of temporal regulation of annexin abundance and positioning. The amount of annexin is more significant at night (when the leaf droops) and the protein is largely cytosolic, whilst in the morning (when the leaf is held up) it has redistributed to the outermost periphery of the motor cells in the pulvinus (Hoshino et al., 2004). Quite what the annexin does in either position remains unknown but its abundance is positively regulated by ABA (Hoshino et al., 2004), which suggests that annexins link stress responses with nyctinastic and possibly seismonastic (touch-induced) movement. Both types of movement involve Ca\(^{2+}\) influx from the apoplast (Campbell and Thomson, 1977), and perhaps this is involved in annexin relocation. *Mimosa* annexin binds F-actin *in vitro* (Hoshino et al., 2004). Actin filaments in *Mimosa* are thought to control pulvinus movement, in conjunction with altered osmotic pressures, through decreased tyrosine phosphorylation of the actin, leading to tissue bending. However, since *Mimosa* annexin distribution does not precisely follow actin distribution *in vivo*, the relationship between the two remains unclear (Hoshino et al., 2004; Kanzawa et al., 2006).

Although there are no reports as yet for involvement of annexins in gravitropism, they are implicated in mediating the resultant differential growth response. Prior to a gravitational stimulus, annexins are asymmetrically distributed in the direction of gravity at the periphery of cells just below the apical meristem of etiolated pea plumules (Clark et al., 2000). Using immunofluorescence, annexins were found to redistribute within 15 min of a gravitational stimulus (and prior to the onset of plumule curvature), occupying a more evenly distributed peripheral position (Clark et al., 2000). This has been interpreted in terms of an annexin involvement in redirecting materials for growth (Clark et al., 2000). In gravistimulated *Arabidopsis* roots, the abundance of AnxAt1 increases in roots (Kamada et al., 2005) and predominates in epidermal cells that would undergo the greatest growth rate to bend the root (Clark et al., 2005b). This distribution largely matched that of polysaccharide secretion, supporting a role for annexins in the gravistimulated growth response (Clark et al., 2005b). In gravistimulated hypocotyls, AnxA2 was detected preferentially in the epidermis that would grow the fastest (Clark et al., 2005b). Determination of how these distributions are caused and link to gravistimulated changes in [Ca\(^{2+}\)]\(_{\text{cyt}}\), actin, and ROS is increasingly within the technical range of plant biologists. Overall, the ability to bind membrane, GTP, and actin suggests the involvement of annexins in (differential) growth and places them downstream of a wide range of signal transduction pathways.

**Responding to stress stimuli and pathogens**

The mechanical stress caused by wind results in short, bushy plants (thigmomorphogenesis). Annexins respond to mechanical stress in *B. dioica* internodes by redistributing from the cytosol to the plasma membrane in parenchyma cells (sampled 30 min after the stimulus; Thonat et al., 1997). The chain of events leading to this remains to be determined. However, mechanical stress is known to elevate [Ca\(^{2+}\)]\(_{\text{cyt}}\) and this could stimulate annexin–plasma membrane association. The significance of annexin relocation is not understood, but as regulators of growth they may govern the radial expansion that results from mechanical stress or could be ‘conditioning’ the plasma membrane for further stress responses.
Cold causes increased annexin expression in poplar leaves (Renault et al., 2006). In wheat, the cold-induced accumulation of the plasma membrane could be involved in sensing or transducing Ca\(^{2+}\) signals or in regulating [Ca\(^{2+}\)\(_{\text{cyt}}\) during signalling or acclimation (Breton et al., 2000). Annexin expression, abundance, and cellular position can respond to osmotic stress, salinity, drought, and ABA (Watkinson et al., 2003; Lee et al., 2004; Buitink et al., 2006; Vandeputte et al., 2007). Alfalfa annexin AnxMs2 mRNA increased during tissue development, but the amount of transcripts was elevated significantly upon NaCl treatment or exogenous ABA (Lee et al., 2006) found that AnxAt1 mRNA abundance is increased by salinity stress. The temporary annexin relocation to membranes in response to ABA and saline stress (Lee et al., 2004) could reflect a role in signalling, or represent their regulation of proteins already in the membrane or annexin-mediated insertion of new proteins to cope with adverse conditions. AnxAt1 transcript accumulates in response to phosphate deprivation (Müller et al., 2007) and in the presence of H\(_2\)O\(_2\) (Gidrol et al., 1996). As H\(_2\)O\(_2\) accumulates in response to phosphate deprivation (Shin et al., 2005), this result suggests that AnxAt1 operates downstream in this stress response. AnxAt1 expression is also up-regulated by salicylic acid, which implicates this annexin in pathogen defence responses (Gidrol et al., 1996). Expression of Arabidopsis AnxAt4, tomato AnxLe34, and tobacco AnxNt12 also increases during pathogen attack but the functional significance remains unknown (Xiao et al., 2001; Truman et al., 2007; Vandeputte et al., 2007). Salinity and ABA also induce AnxNt12 expression, but application of H\(_2\)O\(_2\) does not, suggesting control by distinct signalling pathways and specific annexin function (Vandeputte et al., 2007).

Annexins and reactive oxygen species

Production of ROS is implicated in control of plant (tropic) growth and development, in some cases controlling [Ca\(^{2+}\)\(_{\text{cyt}}\)] (reviewed by Gapper and Dolan, 2006). Stress conditions that cause ROS generation (such as pathogen attack, drought, salinity, cold, hypoxia, and nutritional restriction) also prompt annexin accumulation or relocation to membranes. However, it is as yet unclear whether ROS or elevated [Ca\(^{2+}\)\(_{\text{cyt}}\)] trigger annexin responses. Membrane oxidation increases membrane binding of animal annexins (Balasubramanian et al., 2001). Peroxide can induce the channel-forming vertebrate AnxA5 to be inserted into membranes in vitro, and peroxide-induced Ca\(^{2+}\) influx in vivo in DT40 pre-B cells requires AnxA5 (Kubista et al., 1999). From this it follows that channel-forming plant annexins (such as AnxA1) are candidates for the ROS-activated channels identified in several plant cells (Foreman et al., 2003).

Recently, annexins have been identified as protein components of an M. truncatula plasma membrane lipid raft alongside signalling and redox proteins (Lefebvre et al., 2007). They could, in common with raft-associated animal annexins (Babiychuk and Draeger, 2000), anchor rafts to the actin cytoskeleton (Konopka-Postupolska, 2007). Alternatively, conceivably, raft-associated annexins could function as channels or peroxidases operating in a localized ROS signalling ‘hub’. AnxAt1 is present at the root hair apex (which is thought to harbour lipid rafts; Jones et al., 2006) and as a peroxidase could help regulate the intracellular peroxide generated during polar growth (Foreman et al., 2003). As peroxidases, annexins could regulate peroxide generated as an inter- or intracellular messenger or relay/terminate a signal through peroxide-dependent oxidation. A protective role can also be envisaged. Peroxidase activity of annexins associated with chloroplast RNA polymerase could protect newly synthesized transcripts from oxidation (Pfannschmidt et al., 2000).

Future perspectives

Sensors? Skeletal regulators? Channels? Peroxidases? Plant annexins are potentially multifunctional proteins in vivo involved in membrane dynamics, actin modelling, and [Ca\(^{2+}\)\(_{\text{cyt}}\)] and ROS regulation. Swifter resolution of stimulus-induced annexin relocation to membranes (particularly in single cell paradigms such as the root hair or guard cell) will help secure definitions of function, as will the increasing availability of mutants.

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References


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