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Review

Multifunctional annexins

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ABSTRACT

Annexins are soluble proteins that undergo conditional association or insertion into membranes. Plants contain several isoforms, each of which may be capable of supporting more than one *in vitro* activity such as actin binding, phosphodiesterase activity, peroxidase activity, and cation transport. Enzymatic activities are modulated by lipid binding, Ca^{2+} and S-glutathionylation. A given annexin can occupy diverse positions in cells, including the apoplast and organelles, with membrane association and expression often as a consequence of perception of a stimulus (for example, salinity, nodulation) that may involve reactive oxygen species. The ability to translocate Ca^{2+} *in vitro* identifies annexins as a novel class of plant ion transporters that could account for channel activities in plasma- and endo-membranes and suggests roles in plant signalling and development. Studies on loss of function or overexpressing lines firmly implicate annexins as participating in the regulation of drought and salinity stress responses. How annexins operate *in vivo*, in terms of localisation and protein function now needs to be determined. With several tiers of regulation (space, time, post-translational modification) potentially operating on the soluble and membrane populations, annexins are complex components of plant cell Ca^{2+} networks.

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1. Introduction

Annexins are encoded by a multi-gene family and expressed in plants, vertebrates, invertebrates, fungi and protists [1]. Named from their ability to join objects together, these small (32–36 kDa for plants), soluble proteins are predominantly cytosolic. Depending on local conditions of cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$), pH, lipid composition or membrane voltage they can attach to or insert into plasma- or endo-membranes [1–9]. In, general, it is a rise in $[Ca^{2+}]_{cyt}$ that promotes relocation to membranes and has lead to consideration of annexins as Ca^{2+} sensors or effectors in $[Ca^{2+}]_{cyt}$-driven processes [1,10–12]. Most eukaryotic cells appear to express several annexins at a time. Tracking the transient redistribution of two animal annexins from the cytosol to membranes (following pathogen challenge and elevation of $[Ca^{2+}]_{cyt}$) has shown differential recruitment and transit time to the plasma membrane (PM). This can be related to the annexin's affinity for Ca^{2+} [13]. Understanding the factors controlling annexin relocation to membranes is only the first challenge. What do they do when they get there and how? These are hard questions

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indeed, especially when an annexin may visit more than one membrane on its journey [13] and be capable of more than one function.

Annexins from vertebrates have been investigated in greater detail than other family members. They are implicated in the regulation of endo- and exo-cytosis, the spatial organization of membrane lipids (including rafts), membrane repair, and the linking of membranes to the actin cytoskeleton [1,10–17]. Vertebrate annexins also appear capable of regulating transport proteins involved in determining $[Ca^{2+}]_{cyt}$, such as the sarcoplasmic reticulum ryanodine receptor [1,12]. Annexins from both vertebrates and invertebrates can form Ca^{2+}-permeable ion channels in artificial membranes [2,5–9,18–26]. This implicates them again in $[Ca^{2+}]_{cyt}$ homeostasis and Ca^{2+}-driven signal transduction [1,2]. However, there are no reports demonstrating loss of equivalent transport activity in native membranes of annexin mutants. Lack of such direct evidence greatly restricts the understanding of annexin function. At present, vertebrate annexins are implicated in regulation of heart cell contractile function [27], stress responses such as hypoxia and inflammation [12], and apoptosis [28]. Their association with human pathological states, notably chronic inflammatory conditions [29] and cancer [30], should drive further research on mode of action.

Plant annexin function is increasingly well defined at *in vitro* protein level but relating their apparent multifunctionality to cellular and physiological functions has met with limited success. The complexity of their cellular distributions [3,31,32] and the possibility of redundancy represent a significant challenge to interpreting mutant phenotypes. Despite this, plant annexins are implicated in a wide range of processes including exocytosis, cell elongation, wall synthesis, stress responses, nodulation, and fruit ripening [3,32–40]. Experimental leads have been taken from animal studies but plant annexins form a disparate group within the family and may have divergent functions. Here we review briefly what is known about annexin protein functions and how these may relate to cellular activities. Phylogeny, structure, nucleotide triphosphatase activity, and actin binding have been addressed more fully in a recent review [3]. The emphasis here is on the possible function of plant annexins in regulating $[Ca^{2+}]_{cyt}$ and reactive oxygen species (ROS) as components of signalling pathways.

2. Plant annexin expression and protein distribution

Plants appear to harbour at least two annexins; their expression is dynamic throughout the life cycle and changes in response to abiotic and biotic stress [3,31,32,37,41]. Expression can vary with cell cycle stage [34]. Three peaks of expression of *Nicotiana tobacum* annexins Ntp32.1 and Ntp32.2 have been observed in the cell cycle of tobacco suspension cells. These occurred at the G2/M transition, mitosis, and the G1/S transition [34]. Annexins are consistently found to be associated with growing cells, including root hairs, pollen tubes and cotton fibres [3,31,36,41]. Work on tobacco has shown that different annexins may operate in cell expansion as opposed to cell division. While expression of the Ntp32.1 and Ntp32.2 annexins correlates positively with division, that of another tobacco annexin, VCaB24, correlates positively with cell expansion [34,35]. The locations of these annexins also differ. Ntp32.1 and Ntp32.2 lie beneath the PM and are implicated in exocytosis and wall formation [34] but VCaB24 associates with the developing tonoplast [35]. Thus, identity and position of an annexin may inform us of specific functions.

At the cellular level, it is becoming clear that a given annexin can occupy various positions depending on cell type and incoming stimuli. *Arabidopsis thaliana* has eight annexins [32] and cellular localisation data on Annexin 1 (ANN1At1), the most abundant in this species, are summarised in Table 1. The apparent ability of an annexin, such as AtANN1, to be cytosolic, extracellular, associated with the plasma membrane, tonoplast and organellar membranes complicates the extrapolation of any *in vitro* protein function to the cellular and whole plant level. The residence of a given annexin in multiple cellular locations raises the question of how such apparent targeting is achieved. Immunofluorescence indicates the association of a *Medicago sativa* annexin (ANNMs2) with the nucleolus but no nuclear targeting sequences have been identified [42]. Whether annexins found in the chloroplast stroma have undergone progressive transit through the lipid phase of the envelope or have been translocated through the chloroplast's protein import machinery remains to be determined. That plant annexins can be extracellular (in common with some animal annexins) could be the consequence of an involvement in exocytosis. Many cell wall proteins lack the classic N-terminal signal peptide for secretion but ability to be secreted can be predicted using the SecretomeP program [43]. Two *Arabidopsis* annexins (ANNAt1, ANNAt2) are implicated in exocytosis [44] but are predicted to be secreted despite the absence of the N-terminal peptide [45]. Both these annexins have been detected in cell walls [46,47]. This implies targeting to the outside of the cell rather than "overspill" from a mechanistic involvement in exocytosis.

Advances in sequence analysis may yet help explain the various locations of a cell's annexin complement. Increases in $[Ca^{2+}]_{cyt}$ or pH could be sufficiently discrete in the vicinity of a membrane to recruit a specific annexin, depending on its lipid-binding characteristics. Post-translational modifications may also help determine the sub-cellular distribution of an annexin and its function. ANNAt1 has been detected as having two different masses in leaves [48], supporting the existence of modified isoforms. It can be S-nitrosylated in response to nitric oxide [49] and recent work on drought stress has shown that it is S-glutathionylated *in vivo* in response to abscisic acid (ABA) [39]. Work on wheat annexins has also lead to the conclusion that post-translational modification or interaction with other proteins will determine cellular location and function [50].

Judging from ANN1At, annexins are likely to respond to multiple environmental signals. This annexin alone is responsive to light, gravity, osmotic stress, salinity, peroxide, phosphate

Table 1
Multi-site distribution of *Arabidopsis thaliana* ANN1. ANNAt1 has been detected in multiple locations at whole plant and at a sub-cellular level. Although not an exhaustive survey the table gives some idea of the difficulties that will be faced in placing *in vitro* data on protein function into a physiological or developmental context.

Location	Stimulus/developmental stage	References
Cell wall from cell suspension culture	N/A	[47]
Plasma membrane of cell suspension culture	Constitutive, also flagellin22- and/or xylanase-induced	[87,88]
Leaves and petioles, plasma membrane	N/A	[48,89]
Root plasma membrane	Drought stress	[90]
Vacuoles from cell culture or rosette leaves	N/A	[91,92]
Glyoxysomes of cotyledon	Etiolation	[93]
Chloroplast	N/A	[94–98]
Root apices total soluble protein	Gravitational stimulus increases expression	[99]