Annexins: a novel family of calcium- and membrane-binding proteins in search of a function

Although the annexins have been extensively studied and much detailed structural information is available, their *in vivo* function has yet to be established.

Structure 15 March 1995, **3**:233–237

Annexins are a family of structurally related proteins which share the property of calcium (Ca^{2+})-dependent binding to phospholipids [1–3]. About 13 members have been identified so far on the basis of sequence homology, closely related biochemical properties and phospholipid binding assays. They were found in a number of tissues and cell types in higher and lower eukaryotes including mammals, birds, fish, amphibia, *Drosophila*, *Dictyostelium* and plants [4]. Annexins clearly differ from the 'EF hand' family of Ca^{2+}-binding proteins [5] in their sequence and the geometry of their Ca^{2+}-binding sites as well as in their affinity for Ca^{2+}. With the exception of the eight-repeat annexin VI, the core regions (C termini) of annexins are composed of four highly conserved 70 amino acid repeats, each corresponding to a distinct domain of similar primary sequence. In contrast, the N termini are diverse in sequence and length (ranging from 12–169 residues) and may confer specific functions upon each annexin type [6,7].

Although a clear *in vivo* role for annexins has yet to be determined, a wide range of biological functions has been proposed for them. The best documented of these include aggregation and fusion of membranes and an involvement in endocytosis and exocytosis [8], inhibition of phospholipase A_2 and thus inflammatory effects by blocking the release of arachidonic acid [9], anticoagulation [10], interaction with cytoskeletal proteins [11] and an enzymatic role in inositol phosphate metabolism [12]. Some members of the annexin family are expressed in a growth-dependent manner [13] and are targets for cellular kinases *in vivo* [14]. These results led to the proposal that annexins are involved in differentiation and mitogenesis. Some annexins, in particular I and V, are secreted in certain tissues, although they lack a signal sequence [15,16]. Their anticoagulant and antiinflammatory effects can probably be explained by membrane substrate depletion, because annexins compete with phospholipase A_2 and blood coagulation factors upon binding to the cell membrane, thereby reducing the substrate availability [9,17–19].

Phosphorylation of annexins occurs at the N termini and can modulate their properties. For example, phosphorylated annexin II requires higher Ca^{2+} concentrations for phospholipid binding than the unphosphorylated protein [20], whereas phosphorylation of annexin I reduces the amount of Ca^{2+} required [21,22], and leads to enhanced degradation by proteolysis [23]. Phosphorylation of annexins I and II regulates their ability to cause lipid vesicle aggregation [24,25]. Further post-translational modifications include the glycosylation of annexins I and II [26] and the N-myristoylation of annexin XIII [27]. Annexin II forms a heterotetramer with p11, an 11 kDa protein with homology to the 'EF hand'-related S100 protein family, which is supposed to exist *in vivo* [28,29]. The (annexin II–p11)$_2$ complex displays an enhanced Ca^{2+} sensitivity in phospholipid binding [22] and is able to aggregate and fuse adrenal chromaffin secretory granules at micromolar Ca^{2+} concentrations [30]. Annexins I, V, VI and VII also display voltage-dependent ion channel activity in phospholipid bilayers *in vitro* [31–35] (reviewed in [36]).

Crystal structure of annexin V

To date, the crystal structures of human [37–40], chicken [41] and rat [42] annexin V have been determined, as well as the C-terminal core of human annexin I [43]. Annexin V comprises 320 amino acid residues and is almost entirely α-helical. Each of the four homologous repeats is folded into a compact domain of similar structure comprising five α-helices, wound into a right-handed superhelix. The four domains are arranged in a cyclic array, giving the molecule an overall flat, slightly curved shape with a convex and a concave face (Fig. 1a).

The three main Ca^{2+}-binding sites are located in domains I, II and IV on the convex face of the molecule. They are structurally very similar to the phospholipase A_2 Ca^{2+} site and were therefore suggested to be the phospholipid-binding sites [44,45], but here the similarity between the two proteins ends. The Ca^{2+} is hepta-coordinated by three main-chain carbonyl oxygens, a bidentate carboxylate group from an acidic side chain 38 residues away in the sequence, and additional water molecules [38].

The domains II and III, and I and IV, form tight modules with approximate two-fold symmetry. A very prominent hydrophilic pore lies in the center of the molecule and has been associated with the ion channel conduction pathway [45]. Its framework is a four-helix bundle (formed by helices A and B of domains II and IV) coated with highly conserved charged residues that allows a chain of water molecules to be buried within the central pore.

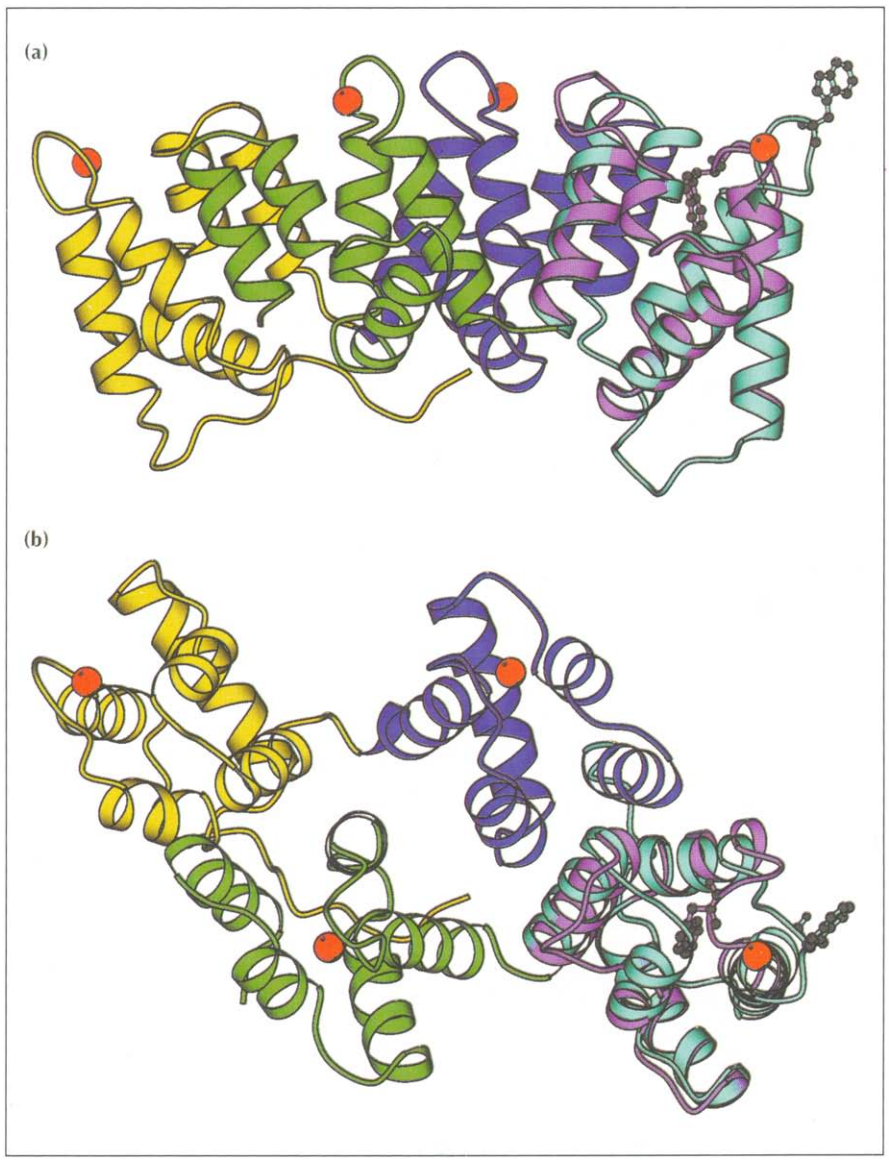

Fig. 1. Superposition of the high-calcium [40] and low-calcium [71] forms of human annexin V, showing the conformational change in domain III (cyan for the high-calcium form and violet for the low-calcium form). The structure is viewed in **(a)** from the side, and in **(b)** from above. Calcium ions are indicated by red spheres in all four domains and each domain is shown in a different color: domain I, yellow; domain II, blue, and domain IV, green. The side chain of Trp187 (shown in ball-and-stick representation) which is normally buried, becomes exposed on the surface by the Ca^{2+}-induced conformational change. (Figure produced using MOLSCRIPT [72].)

Recently, a novel feature of the annexin V structure has been revealed by analysis of crystal forms obtained under modified Ca^{2+} concentration conditions [34,35,40,42]. In a Ca^{2+}-induced conformational change, the normally buried Trp187-containing loop in domain III becomes exposed on the molecular surface (Fig. 1). This conformational change leads to the formation of an additional strong Ca^{2+}-binding site in this domain. The Ca^{2+} has a sulfate ion from the precipitating agent as a ligand, presumably mimicking the phosphoryl group of a membrane phospholipid [34,35,40]. The same conformational change has been observed in solution by fluorescence studies in the presence of calcium [46,47] and phospholipids [48,49], suggesting a direct contact between the exposed tryptophan and the ester-bond region of the membrane.

Mutational studies on annexins

The first mutational studies on annexins were mainly aimed at probing their Ca^{2+}- and phospholipid-binding properties. Site-directed mutagenesis of annexin II predicted altered Ca^{2+}-binding properties of domains I and III compared with annexin V [50]. In other experiments, the p11-binding site for annexin II–p11 heterotetramer formation was localized to the N-terminal part of annexin II [51]. Mutagenesis of the conserved Ca^{2+}-binding loop in domain II of annexin I resulted in modified Ca^{2+} affinities and self-association properties [52]. Experiments with a chimeric protein consisting of the annexin V α-helical core and the annexin I N terminus indicate that the annexin membrane-interaction properties might be influenced by the N terminus, because the chimera was capable of aggregating phospholipid vesicles like annexin I [53].

Mutational studies were also employed to achieve a better understanding of the ion channel properties of annexin V, the first ion channel protein defined to high resolution [34–36]. The replacement Glu95→Ser in the center of the pore region resulted in significantly modified Ca^{2+} channel properties (Fig. 2), indicating that Glu95 is a crucial component of the ion-selectivity filter [34,35].

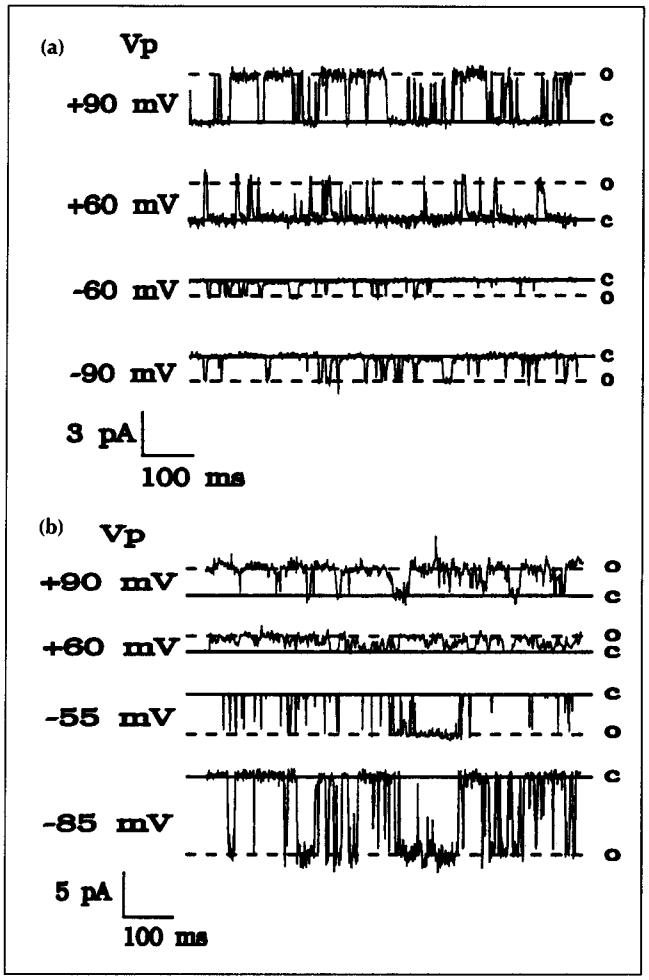

Fig. 2. Single channel currents for **(a)** wild-type annexin V and **(b)** the Glu95→Ser mutant incorporated into acidic phospholipid bilayers at different pipette potentials (Vp); O, open state; C, closed state. The mutation resulted in a lower single-channel conductance of Ca^{2+}, a strongly increased conductance for Na^+ and K^+, as well as complete loss of selectivity for Ca^{2+} *versus* Na^+ [34,35].

The disruption of a conserved salt bridge in the upper part of the pore causes a complete loss of the voltage dependence of channel gating [36]. These results support the assumption that the salt bridges within the pore function as voltage-sensing gates, as side-chain rearrangement is required for ion permeation [54]. The removal, by mutagenesis, of surface charges distant from the ion pore causes modified conductance patterns, which may be attributable to the modification of the dynamic properties of the molecule [36]. Indeed, the dynamics of the molecule can change upon Ca^{2+} binding [55], while the intermodule angle (i.e., the angle between domains II/III and domains I/IV) which directly influences the width of the central pore has been shown to vary in different crystal structures of annexin V [34,35,37,39,40]. The equilibrium between conformers with different intermodule angle is sensitive to the macro-dipole of the molecule represented by the charge distribution within the two modules [35,36].

Annexin–membrane interaction

Several water-soluble, channel-forming proteins are proposed to undergo profound structural changes upon integration into the membrane to attain their conducting state [56]. The annexins are atypical in this respect, as all experimental data available to date reveal that they remain peripherally bound [57,58].

Annexins bind Ca^{2+} with K_d values in the millimolar range, whereas the binding of annexins to phospholipids has a K_d around 10^{-10} M at Ca^{2+} concentrations of ~10–100 μM [2]. Annexin V forms Ca^{2+}-induced dimers in aqueous solution [18,55], while in the presence of phospholipids, trimers, hexamers and higher aggregates have been observed [59]. Self-association has been reported for various annexins on membrane surfaces [60,61].

The membrane-bound structures of annexin V [58,60,62,63] and annexin VI [64,65] have been investigated by electron microscopy (EM). More recently, two-dimensional crystals of annexin V bound to phospholipid monolayers have been analyzed (Fig. 3) allowing the three-dimensional structure of these complexes to be reconstructed [58]. These results show that membrane binding is mediated by the convex surface of the molecule that contains the Ca^{2+}-binding sites [34,58]. Comparison of this structure with the high-resolution crystal structure shows that the molecule has the same

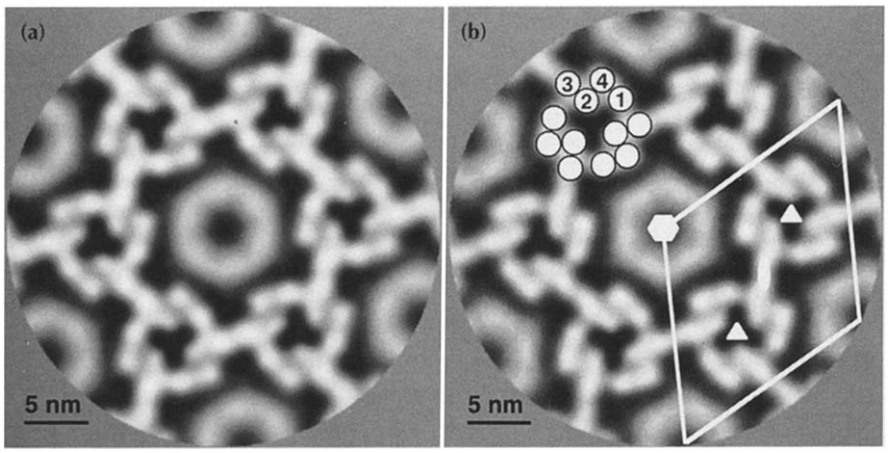

Fig. 3. Electron micrographs of membrane-bound **(a)** wild-type annexin V and **(b)** a Glu95→Ser mutant at 20 Å resolution. The images are views from the membrane side after image averaging. The negatively stained crystals are isomorphous, with a p6 lattice and a periodicity of 18.3 nm. The unit cell, outlined by the white diamond in (b), contains two trimers of annexin V and a central ring located on the hexad, which is a translationally and rotationally disordered annexin V trimer [34,58].

overall domain structure in its membrane-bound state as in solution, but that the relative orientation of the modules (II,III) and (I,IV) is slightly changed so that the Ca^{2+}-binding sites in all four domains (including the recently observed binding site in domain III [34, 35,40,42]) become coplanar with the membrane. The EM reconstruction indicates that annexin V does not penetrate the membrane upon binding, because the height of the molecule, as estimated from the EM measurements, is the same as its crystallographically determined size [58]. An analogous observation had been made in a small-angle neutron scattering study showing the arrangement of protein monolayers on phospholipid vesicles [66].

An important question remains. How can peripherally bound annexin V form highly conducting ion channels through membranes? As suggested by Karshikov *et al.* [54], the annexin V electrostatic potential may cause a strong local gradient at the protein–membrane interface generating a pore through the membrane by a microscopic phenomenon comparable to electroporation. The characteristics of the membrane in the protein-bound state are indeed considerably modified as shown by a variety of experiments [67–70], but further detailed studies are needed. It is still a matter of debate as to whether long-lived, water-filled pores exist, or whether the pores are formed by transiently disordered, less tightly packed areas [3,36].

Acknowledgement: We thank Robert Huber for his interest and helpful discussions.

References

1. Moss, S.E. (Ed.) (1992). *The Annexins.* Portland Press, London.
2. Raynal, P. & Pollard, H.B. (1994). Annexins: the problem of assessing the biological role for a gene family of multifunctional calcium- and phospholipid-binding proteins. *Biochim. Biophys. Acta* **1197**, 63–93.
3. Swairjo, M.A. & Seaton, B.A. (1994). Annexin structure and membrane interactions: a molecular perspective. *Annu. Rev. Biophys. Biomol. Struct.* **23**, 193–213.
4. Crumpton, M.J. & Dedman, J.R. (1990). Protein terminology tangle. *Nature* **345**, 212.
5. Kretsinger, R.H. (1980). Structure and evolution of calcium-modulated proteins. *CRC Crit. Rev. Biochem.* **8**, 119–174.
6. Crompton, M.R., Moss, S.E. & Crumpton, M.J. (1988). Diversity in the lipocortin family. *Cell* **55**, 1–3.
7. Barton, G.J., Newman, R.H., Freemont, P.S. & Crumpton, M.J. (1991). Amino acid sequence analysis of the annexin super-gene family of proteins. *Eur. J. Biochem.* **198**, 749–760.
8. Creutz, C.E. (1992). The annexins and endocytosis. *Science* **258**, 924–931.
9. Davidson, F.F., Lister, M.D. & Dennis, E.A. (1990). Binding and inhibition studies on lipocortins using phosphatidylcholine vesicles and phospholipase A_2 from snake venom, pancreas and a macrophage-like cell line. *J. Biol. Chem.* **265**, 5602–5609.
10. Römisch, J., Schorlemmer, U., Fickenscher, K., Pâques, E.P. & Heimburger, N. (1990). Anticoagulant properties of placenta protein-4 (annexin V). *Thromb. Res.* **60**, 355–366.
11. Mangeat, P.-H. (1988). Interaction of biological membranes with the cytoskeletal framework of living cells. *Biol. Cell* **64**, 261–281.
12. Ross, T.S., Tait, J.T. & Majerus, P.W. (1990). Identity of inositol 1,2-cyclic phosphate 2-phosphohydrolase with lipocortin III. *Science* **248**, 605–607.
13. Schlaepfer, D.D. & Haigler, H.T. (1990). Expression of annexins as a function of cellular growth-state. *J. Cell Biol.* **111**, 229–238.
14. Moss, S.E., Edwards, H.C. & Crumpton, M.J. (1991). Diversity in the annexin family. In *Novel Calcium-Binding Proteins.* (Heizmann, E.W., ed), pp. 535–566, Springer-Verlag, Berlin.
15. Christmas, P., Callaway, J., Fallon, J., Jones, J. & Haigler, H.T. (1991). Selective secretion of annexin I, a protein without a signal sequence, by the human prostate gland. *J. Biol. Chem.* **266**, 2499–2507.
16. Pfäffle, M., Ruggiero, F., Hofman, H., Fernandez, M.P., Selmin, O. & von der Mark, K. (1988). Biosynthesis, secretion and extracellular localization of anchorin-cII, a collagen-binding protein of the calpactin family. *EMBO J.* **7**, 2335–2342.
17. Davidson, F.F., Dennis, E.A., Powell, M. & Glenney, J.R. (1987). Inhibition of phospholipase A_2 by lipocortins and calpactins — an effect of binding to substrate phospholipids. *J. Biol. Chem.* **262**, 1698–1705.
18. Ahn, N.G., Teller, D.C., Bienkowski, M.J., McMullen, B.A., Lipkin, E.W. & de Haen, C. (1988). Sedimentation equilibrium analysis of five lipocortin-related phospholipase A_2 inhibitors from human placenta. *J. Biol. Chem.* **263**, 18657–18663.
19. Russo-Marie, F. (1992). Annexins, phospholipase A_2 and the glucocorticoids. In *The Annexins.* (Moss, S.E., ed), pp. 35–46, Portland Press, London.
20. Powell, M.A. & Glenney, J.R. (1987). Regulation of calpactin-I phospholipid binding by calpactin-I light-chain binding and phosphorylation by $p60^{v\text{-}src}$. *Biochem. J.* **247**, 321–328.
21. Schlaepfer, D.D. & Haigler, H.T. (1987). Characterization of Ca^{2+}-dependent phospholipid binding and phosphorylation of lipocortin I. *J. Biol. Chem.* **262**, 6931–6937.
22. Ando, Y., Imamura, S., Hong, Y.M., Owada, M.K., Kakunaga, T. & Kannagi, R. (1989). Enhancement of calcium sensitivity of lipocortin I in phospholipid binding induced by limited proteolysis and phosphorylation at the amino terminus as analyzed by phospholipid affinity column chromatography. *J. Biol. Chem.* **264**, 6948–6955.
23. Chuah, S.Y. & Pallen, C.J. (1989). Calcium-dependent and phosphorylation stimulated proteolysis of lipocortin I by an endogenous A431 cell-membrane protease. *J. Biol. Chem.* **264**, 21160–21166.
24. Wang, W. & Creutz, C.E. (1994). Role of the amino-terminal domain in regulating interactions of annexin I with membranes: effects of amino-terminal truncation and mutagenesis of the phosphorylation sites. *Biochemistry* **33**, 275–282.
25. Johnstone, S.A., Hubaishy, I. & Waisman, D.M. (1992). Phosphorylation of annexin II tetramer by protein kinase C inhibits aggregation of lipid vesicles by the protein. *J. Biol. Chem.* **267**, 25976–25981.
26. Goulet, F., Moore, K.G. & Sartorelli, A.C. (1992). Glycosylation on annexin I and annexin II. *Biochem. Biophys. Res. Commun.* **188**, 554–558.
27. Wice, B.M. & Gordon, J.I. (1992). A strategy for isolation of cDNAs encoding proteins affecting human intestinal epithelial cell growth and differentiation: characterization of a novel gut-specific N-myristoylated annexin. *J. Cell Biol.* **116**, 405–422.
28. Gerke, V. & Weber, K. (1985). Calcium-dependent conformational changes in the 36-kDa subunit of intestinal protein I related to the cellular 36-kDa target of Rous sarcoma virus tyrosine kinase. *J. Biol. Chem.* **260**, 1688–1695.
29. Weber, K. (1992). Annexin II: interaction with p11. In *The Annexins.* (S.E. Moss, ed), pp. 61–68, Portland Press, London.
30. Drust, D.S. & Creutz, C.E. (1988). Aggregation of chromaffin granules by calpactin at micromolar levels of calcium. *Nature* **331**, 88–91.
31. Pollard, H.B. & Rojas, E. (1988). Ca^{2+}-activated synexin forms highly selective, voltage-gated Ca^{2+} channels in phosphatidylserine bilayer membranes. *Proc. Natl. Acad. Sci. USA* **85**, 2974–2978.
32. Rojas, E., Pollard, H.D., Haigler, H.T., Parra, C. & Burns, A.L. (1990). Calcium-activated endonexin II forms calcium channels across acidic phospholipid membranes. *J. Biol. Chem.* **265**, 21207–21215.
33. Pollard, H.B., *et al.*, & Rojas, K. (1992). Calcium channel and membrane fusion activity of synexin and other members of the annexin gene family. *Biophys. J.* **62**, 15–18.
34. Berendes, R., Voges, D., Demange, P., Huber, R. & Burger, A. (1993). Structure–function analysis of the ion channel selectivity filter in human annexin V. *Science* **262**, 427–430.
35. Burger, A., Voges, D., Demange, P., Perez, C.R., Huber, R. & Berendes, R. (1994). Structural and electrophysiological analysis of annexin V mutants. *J. Mol. Biol.* **237**, 479–499.
36. Demange, P., *et al.*, & Huber, R. (1994). Annexin V: the key to understanding ion selectivity and voltage regulation? *Trends Biochem. Sci.* **19**, 272–276.
37. Huber, R., Römisch, J. & Pâques, E. (1990). The crystal and molecular structure of human annexin V, an anticoagulant protein that binds to calcium and membranes. *EMBO J.* **9**, 3867–3874.
38. Huber, R., Schneider, M., Mayr, I., Römisch, J. & Pâques, E.-P. (1990). The calcium binding sites in human annexin V by crystal structure analysis at 2.0 Å resolution: implications for membrane binding and calcium channel activity. *FEBS Lett.* **275**, 15–21.

39. Lewit-Bentley, A., Morera, S., Huber, R. & Bodo, G. (1992). The effect of metal binding on the structure of annexin V and implications for membrane binding. *Eur. J. Biochem.* **210**, 73–77.
40. Sopkova, J., Renouard, M. & Lewit-Bentley, A. (1993). The crystal structure of a new high-calcium form of annexin V. *J. Mol. Biol.* **234**, 816–825.
41. Bewley, M.C., Boustead, C.M., Walker, J.H., Waller, D.A. & Huber, R. (1993). Structure of chicken annexin V at 2.25 Å resolution. *Biochemistry* **32**, 3923–3929.
42. Concha, N.O., Head, J.F., Kaetzel, M.A., Dedman, J.R. & Seaton, B.A. (1993). Rat annexin V crystal structure: Ca^{2+}-induced conformational changes. *Science* **261**, 1321–1324.
43. Weng, X., Luecke, H., Song, I.S., Kang, D.S., Kim, S.-H. & Huber, R. (1993). Crystal structure of human annexin I. *Protein Sci.* **2**, 448–458.
44. Thunissen, M.M.G., *et al.*, & Verhej, H.M. (1990). X-ray structure of phospholipase A_2 complexed with a substrate-derived inhibitor. *Nature* **347**, 689–691.
45. Huber, R., *et al.*, & Pâques, E. (1992). Crystal and molecular structure of human annexin V after refinement: implications for structure, membrane binding and ion channel formation of the annexin family of proteins. *J. Mol. Biol.* **223**, 683–704.
46. Meers, P. & Mealy, T. (1993). Relationship between annexin V tryptophan exposure, calcium and phospholipid binding. *Biochemistry* **32**, 5411–5418.
47. Sopkova, J., Gallay, J., Vincent, M., Pancoska, P. & Lewit-Bentley, A. (1994). The dynamic behaviour of annexin V as a function of calcium ion binding: a circular dichroism, UV absorption, and steady-state and time-resolved fluorescence study. *Biochemistry* **33**, 4490–4499.
48. Meers, P. (1990). Location of tryptophans in membrane-bound annexins. *Biochemistry* **29**, 3325–3330.
49. Meers, P. & Mealy, T. (1994). Phospholipid determinants for annexin V binding sites and the role of tryptophan 187. *Biochemistry* **33**, 5829–5837.
50. Jost, M., Thiel, C., Weber, K. & Gerke, V. (1992). Mapping of three unique Ca^{2+}-binding sites in human annexin II. *Eur. J. Biochem.* **207**, 923–930.
51. Johnsson, M., Marriontt, G. & Weber, K. (1988). P36, the major cytoplasmic substrate of src tyrosine protein kinase, binds to its p11 regulatory subunit via a short amino-terminal amphipathic helix. *EMBO J.* **7**, 2435–2442.
52. Travé, G., *et al.*, & Liautard, J.-P. (1994). Site-directed mutagenesis of a calcium binding site modifies specifically the different biochemical properties of annexin I. *Protein Eng.* **7**, 689–696.
53. Hoekstra, D., Buist-Arkema, R., Klappe, K. & Reutelingsperger, C.P.M. (1993). Interaction of annexins with membranes: the N-terminus as a governing parameter as revealed with a chimeric annexin. *Biochemistry* **32**, 14194–14202.
54. Karshikov, A., Berendes, R., Burger, A., Cavalié, A., Lux, H.D. & Huber, R. (1992). Annexin V membrane interaction: an electrostatic potential study. *Eur. Biophys. J.* **20**, 337–344.
55. Neumann, J.-M., Sanson, A. & Lewit-Bentley, A. (1994). Calcium-induced changes in annexin V behaviour in solution as seen by proton NMR spectroscopy. *Eur. J. Biochem.* **225**, 819–825.
56. Parker, M.W., Pattus, F., Tucker, A.D. & Tsernoglou, D. (1989). Structure of the membrane-pore-forming fragment of colicin A. *Nature* **337**, 93–96.
57. Andree, H.A.M., Reutelingsperger, C.P.M., Hauptman, R., Hemker, H.C., Hermens, W.T. & Willems, G.M. (1990). Binding of vascular anticoagulant a (VACa) to planar phospholipid bilayers. *J. Biol. Chem.* **265**, 4923–4928.
58. Voges, D., Berendes, R., Burger, A., Demange, P., Baumeister, W. & Huber, R. (1994). Three-dimensional structure of membrane-bound annexin V — a correlative electron microscopy–X-ray crystallography study. *J. Mol. Biol.* **238**, 199–213.
59. Concha, N.O., Head, J.F., Kaetzel, M.A., Dedman, J.R. & Seaton, B.A. (1992). Annexin V forms calcium-dependent trimeric units on phospholipid vesicles. *FEBS Lett.* **314**, 159–162.
60. Mosser, G., Ravanat, C., Freyssinet, J.-M. & Brisson, A. (1991). Subdomain structure of lipid-bound annexin V resolved by electron image analysis. *J. Mol. Biol.* **217**, 241–245.
61. Pigault, C., Follenius-Wund, A., Schmutz, M., Freyssinet, J.-M. & Brisson, A. (1994). Formation of two-dimensional arrays of annexin V on phosphatidylserine-containing liposomes. *J. Mol. Biol.* **236**, 199–208.
62. Brisson, A., Moser, G. & Huber, R. (1991). Structure of soluble and membrane-bound human annexin V. *J. Mol. Biol.* **220**, 199–203.
63. Newman, R.H., Leonard, K. & Crumpton, M.J. (1991). 2D crystal forms of annexin IV on lipid monolayers. *FEBS Lett.* **279**, 21–24.
64. Newman, R., Tucker, A.D., Ferguson, C., Tsernoglou, D., Leonard, K. & Crumpton, M.J. (1989). Crystallization of p68 on lipid monolayers and as three-dimensional single crystals. *J. Mol. Biol.* **206**, 213–219.
65. Driessen, H.P.C., Newman, R.H., Freemont, P.S. & Crumpton, M.J. (1992). A model of the structure of human annexin VI bound to lipid monolayers. *FEBS Lett.* **306**, 75–79.
66. Ravanat, C., Torbet, J. & Freyssinet, J.-M. (1992). A neutron solution scattering study of annexin V and its binding to lipid vesicles. *J. Mol. Biol.* **226**, 1271–1278.
67. Swairjo, M.A., Roberts, M.F., Campos, M.-B., Dedman, J.R. & Seaton, B.A. (1994). Annexin V binding to the outer leaflet of small unilamellar vesicles leads to altered inner-leaflet properties: ^{31}P- and ^{1}H-NMR studies. *Biochemistry* **33**, 10944–10950.
68. Bazzi, M.D. & Nelsenstuen, G.L. (1991). Extensive segregation of acidic phospholipids in membranes induced by protein kinase C and related proteins. *Biochemistry* **30**, 7961–7969.
69. Meers, P., Daleke, D., Hong, K. & Papahadjopoulous, D. (1991). Interactions of annexins with membrane phospholipids. *Biochemistry* **30**, 2903–2908.
70. Gilmanshin, R., Creutz, C.E. & Tamm, L.K. (1994). Annexin IV reduces the rate of lateral lipid diffusion and changes the fluid phase structure of the lipid bilayer when it binds to negatively charged membranes in the presence of calcium. *Biochemistry* **33**, 8225–8232.
71. Lewit-Bentley, A., Bentley, G.A., Favier, B., L'Hermite, G. & Renouard, M. (1994). The interaction of metal ions with annexin V: a crystallographic study. *FEBS Lett.* **345**, 38–42.
72. Kraulis, P.J. (1991). MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.* **24**, 946–950.

Susanne Liemann, Max-Planck-Institut für Biochemie, Am Klopferspitz 18a, 82152 Martinsried, Germany. Anita Lewit-Bentley, LURE, Centre Universitaire Paris-Sud, Bâtiment 209D, 91405 Orsay, France.