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Minireview

Annexins – multifunctional, calcium-dependent, phospholipid-binding proteins*

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Nomenclature and general properties of annexins

Annexins belong to the superfamily of structurally related intracellular proteins that bind to membranous anionic phospholipids in the presence of micromolar concentration of free Ca^{2+}. In the literature, the generic term "annexin" has been adopted by most investigators in an effort to rationalize the nomenclature; however, synonymous names are often used, such as calpactins, lipocortins, chromobindins, endonexins, calcimedins, calelectrins, placental anticoagulant proteins or others (for recent reviews see [1 - 3]). These names arose usually from biological source of purification of annexins (e.g. calelectrins and chromobindins) or from their functional properties (e.g. lipocortins and calpactins) [4, 5].

All annexins are monomeric proteins, with the exception of annexin II which, *in vivo*, is usually associated with another polypeptide, p11. Together, they form a heterotetramer, such that two molecules of M_r 36 000 (true annexin, so-called "heavy chain") bind noncovalently to a p11 dimer (so-called "light chain") [1].

In the presence of phosphatidylserine, phosphatidylinositol, or other anionic phospholipids, affinity of annexins for calcium ions is greatly enhanced, therefore annexins may bind to the plasma membrane at physiological concentration of intracellular Ca^{2+} [4, 6 - 8].

In view of the low Ca^{2+} affinity of some of annexins in the absence of membrane phospholipids it is possible that lipid binding directly affects the coordination of Ca^{2+}, so that affinity for both ion and lipid is increased in the ternary complex [9]. For such an interaction the third repeat unit of annexins is probably responsible [10] (see also the "Structural relationships" chapter). In addition, it was found that calcium ions at micromolar concentrations induce a marked decrease in the order parameter of liposome membranes made of the phosphatidylserine and phosphatidylcholine. This effect was completely abolished by annexin IV and VI, probably as a result of formation of calcium "bridges" connecting the phosphatidylserine and annexins [11].

Despite of binding to negatively charged phospholipids in a Ca^{2+}-dependent manner, members of the annexin family possess several important functions and interesting features. Lipocortins inhibit phospholipase A_2 [1, 2, 12 - 14] and influence blood coagulation [1, 2, 15]. Chromobindins promote aggregation of chromaffin granules and their fusion with membranes, as well as with artificial phospholipid vesicles [8, 16, 17]. Annexins are the major substrates of oncogene and growth factor receptor tyrosine kinases [1, 4, 14, 18]. Five members of the annexin family (I, II and IV - VI) have been shown to bind qualitatively F-actin [1, 18]; among them annexins I and II bundle actin

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filaments [1, 5, 19]. In addition to interactions with other proteins, some annexins have been reported to self-associate in the presence of calcium ions, either on or off the phospholipid membrane [20]. Last but not least, annexins V and VII can form a voltage-dependent Ca^{2+} channel in phospholipid bilayer and natural membrane [21, 22].

An updated nomenclature of annexin family members is presented in Table 1, while their major properties, including phospholipid specificity and calcium ion binding, are summarized in Table 2.

Tissue distribution and localization within a cell

Annexins are widely distributed among different eukaryotic species including mammals, fish, plants and insects, where they have been detected in many cell types and various tissues [1, 2, 4, 7, 26, 28, 29]. This may be taken as evidence for their great biological importance in many organisms. In addition, annexins are abundant intracellular proteins that are expressed up to 1% (in placenta up to 2% [30]) of the total cell protein in all vertebrate cell extracts examined to date [4]. They are localized on the cytoplasmic side of plasma membrane, sometimes in association with the cytoskeleton [6, 7, 18, 31, 32]. Annexin IV is partially membrane bound and may be associated with endoplasmic reticulum [4, 6]. In electric organ of *Torpedo marmorata*, this protein is also associated with intracellular membranes and is concentrated at the cytoplasmic face of synaptic vesicles [4, 6, 18]. There is evidence that annexins V and VI may behave like membrane integral proteins [33]. Annexin II (in monomeric form) [34] and the recently discovered annexin CAP-50 [35], have been found within the nuclear matrix and not in the cytoplasm.

Structural relationships

Although relative molecular mass of annexins vary between 28000 - 39000 (annexins I - V, VIII, X, XII) and 67000 - 73000 (annexin VI), all members of the family are characterized by a well defined, similar structure [1, 9, 36]. It consists of a core and N-terminal domains. Within the core domain there are Ca^{2+} and phospholipid-binding sites with structurally conserved four repeats of approx. 70 amino-acid residues each [1, 9, 23] (except for annexin VI which contains related eight repeats [37]). These repeats exhibit a high degree of internal sequence similarity, which is also high among different members of this protein family [38]. All annexins display a high degree of homology (40 - 60%, see also Table 3) [4, 9, 10, 23], first described by Geisow *et al.* [39], who identified a 17-amino-acid consensus sequence: Lys-Gly-h-Gly-Thr-Asp-Glu-x-x-Leu-Ile-p-Ile-Leu-Ala-p-Arg (h – hydrophobic residue, p – polar residue and x – variable residue) present in each of the repeats forming the core of annexins. It is worth mentioning that there are no statistically significant differences between the repeats in annexins examined to date. Moreover, the search made in this protein sequence databases (3,500 sequences) failed to reveal any significant homology between the sequence listed above and that of any known protein [39]. It is generally believed that a highly conserved stretch of 17 amino-acid residues, known as the endonexin fold (or annexin fold), is involved in calcium ions and phospholipid binding [1, 4, 9, 39]. It should be stressed that no sequence homologous to the EF-hand sequence of Ca^{2+}-binding proteins (S-100 superfamily of proteins) can be found in the primary structure of annexins, and this implies that their Ca^{2+}-binding structure is different [1, 3, 4, 7, 40].

Secondary structure prediction of 88 conserved repeat regions from the annexin supergene family led to following conclusions:

- i) the annexin repeats fall into four families, 1 through 4, respectively. Repeats 5 - 8 of annexin VI fall into the same four families;
- ii) the repeat 3 family exhibits the greatest diversity and a rather small similarity to the repeat 1, 2 and 4 families ;
- iii) the secondary structure of the annexin repeat is predicted to consist of five helices, and patterns of conserved hydrophobic amino acids are consistent with one face of a helix packing against the protein core in predicted helices;
- iv) the physico-chemical properties at 22 aligned positions are highly conserved in all repeats;
- v) the distinct secondary structure of repeat 3 suggests an important structural and functional role of this region in the annexin molecule [38].

Recently, a number of different members of the annexin family have been crystallized and

Table 1

Nomenclature of annexins

PAP, placental anticoagulant protein; PP, placental protein; GIF, glycosylation-inhibiting factor; IBC, inhibitor of blood coagulation; VAC, vascular anticoagulant; CaBP, Ca^{2+}-binding protein

Annexin	Previous terminology	Apparent M_r ($\times 10^{-3}$)	Source of isolation	Apparent pI
I	lipocortin I calpactin II p35, GIF chromobindin 9/6 lipomodulin	35 - 40	intestine epithelial cells, lung, placenta, adrenal medulla	6.5 - 6.8
II	lipocortin II calpactin I▲ p36, p33 chromobindin 8 protein I, PAP-IV	33 - 39	intestine epithelial cells, lung, lymphocytes, placenta, adrenal medulla	7.2 - 7.9
III	lipocortin III 35-α calcimedin PAP-III calphobindin III	35	placenta, smooth muscles	5.8 - 6.2
IV	lipocortin IV 35-β calcimedin p28 chromobindin 4 protein II PAP-II, PP4-X endonexin I 32.5 calelectrin	28 - 33	liver, lymphocytes, adrenal medulla, intestine, placenta, pancreas, smooth muscles, electric organ of *Torpedo marmorata*	5.4 - 5.8
V	lipocortin V 35-γ calcimedin chromobindin 5/7 VAC-α, IBC PAP-I, PP4 endonexin II 35K calelectrin calphobindin I anchorin CII CaBP33, CaBP37	33 - 37	smooth muscles, placenta, brain, heart, adrenal medulla, electric organ of *Torpedo marmorata*	4.5 - 5.2
VI	lipocortin VI 67K calcimedin p68, p70, 73K chromobindin 20 protein III 67K calelectrin calphobindin II synhibin	67 - 73	liver, smooth muscles, lymphocytes, adrenal medulla, placenta, brain, heart, aorta, electric organ of *Torpedo marmorata*	5.7 - 6.4
VII	lipocortin VII chromobindin 11 synexin I synexin II	45 - 56	adrenal medulla, *Dictyostelium discoideum*	5.7 - 7.0
VIII	lipocortin VIII VAC-β	33 - 36	placenta	5.2 - 5.8
IX	–	n.d.	*Drosophila melanogaster**	n.d.

X	–	35	*Drosophila melanogaster**	n.d.
XI	–	56	mammalian chondrocytes*	n.d.
XII	–	33	*Hydra vulgaris**	n.d.

Data were taken from [1, 2, 4, 5, 18, 23 - 25]; ▲heavy chain; *identified by cDNA cloning and sequence analysis (annexins IX [26] and XI [27]), and by using recombinant protein (annexin X [26]); n.d. - not determined

Table 2

Phospholipid specificity and Ca^{2+} binding by annexins

PL, phospholipid; PS phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PA, phosphatidic acid

Annexin	Phospholipid class	Ca^{2+} binding	
		Number of binding sites	K_d (mM)
I	PS	1 (–PS) 4 (+PS) 2 (+PS)	 7.5×10^{-2} 10^{-2}
II monomer	PS, PI	2 (+PS/PI) 4	4.5×10^{-3} 0.5×10^{-3} (+PS) 0.5 (–PS)
$p36_2 11_2$*	PS		1.3×10^{-3} (+PS) 0.5 (–PS) 0.1
III	PE, PI, PS		
IV	PI, PE, PA		0.4×10^{-3}
V	PS	4	0.1 (+PS) 0.5 (–PS)
VI	PS, PE, PI, PA	1 (–PL) 1 (–PS) 8 (+PS)	1.2×10^{-3} 2×10^{-2} 0.4×10^{-3} 10^{-3}
VII	PS		
VIII	PS		

Data were taken from [1, 4, 6]; information about annexins IX - XII is not available; *heterotetramer

Table 3

Sequence homology between different species of annexins

Sequences of human annexins I - III, V and VI, and porcine annexin IV were compared and the percentage of sequence identities among various annexins shown (from [36]). VIa and VIb indicate the amino- and carboxyl-terminal halves of annexin VI, respectively

	Annexins						
	I	II	III	IV	V	VIa	VIb
I		54	49	46	43	43	41
II	54		48	50	46	46	43
III	49	48		53	49	51	49
IV	46	50	53		58	54	51
V	43	46	49	58		57	48
VIa	43	46	51	54	57		45
VIb	41	43	49	51	48	45	

three-dimensional structural analysis has been initiated. These include: annexin V from rat kidney [41] and human vascular tissue [42, 43], annexin IV from chicken liver [44] and human placenta [45], and annexin VI from human placenta [46]. It is believed that this will allow detailed structural comparison of different annexins and help to explain their different physical properties, in terms of Ca^{2+} affinity and phospholipid specificity.

In all annexins the N-terminal tail is relatively short (between 8 and 39 amino-acid residues), resulting in their relative molecular masses being 28000 - 39000 for 4-repeat annexins, except for annexin VII (synexins) with M_r of 45000 - 56000 [2, 4, 47], and 67000 - 73000 for the 8-repeat annexins [1, 4] (Fig. 1). The tail probably represents an important regulatory region since, at least in some annexins, it harbors the phosphorylation site for different protein kinases involved in signal transduction, including protein kinases: protein kinase C [1, 5, 51], cAMP-dependent [1], calmodulin-dependent [1], and the oncogene [1, 4, 5, 12] and the epidermal growth factor receptor-related tyrosine [1, 4, 5, 12] kinases (Table 4). In addition, the N-terminal portion is the site of specific interactions with other proteins: F-actin, non-erythroid spectrin (fodrin), collagen, S-100, and p11 [1, 4, 28, 53, 55]. The N-terminal domain of annexin VII has an unique sequence that is much longer (167 amino-acid residues) and more hydrophobic than the N-terminal domains of the other annexins [1, 47].

In conclusion, analysis of amino-acid sequences of 22 annexins suggests that they have evolved from a common gene precursor for a single-repeat preprotein, which then underwent a series of gene duplications, giving as a result four- and eight-repeat annexins [9, 37, 38] (Fig. 2).

Physiological role of annexins

The biological function of annexins is still unknown, and this problem creates an interesting challenge for investigators. Moreover, there are

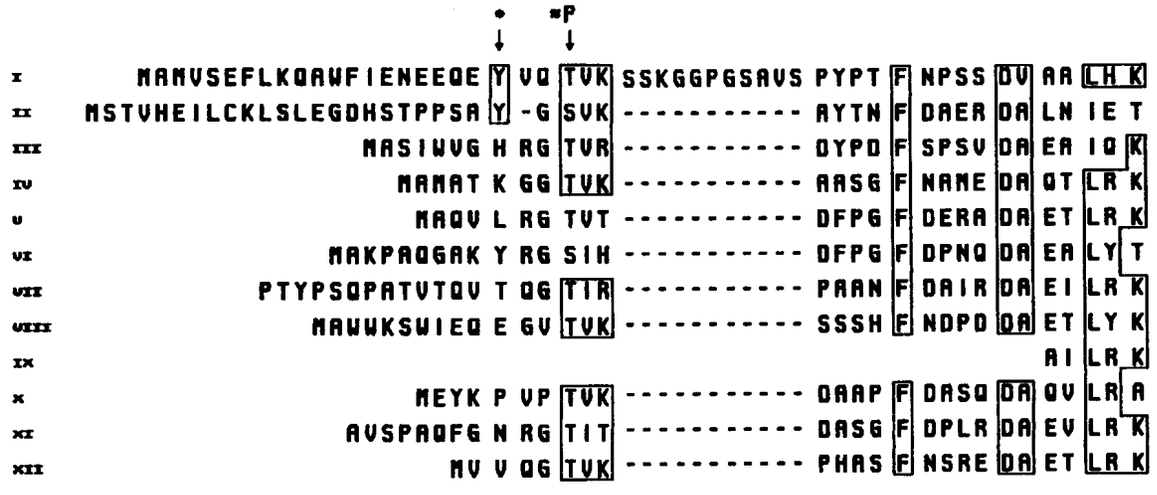

Fig. 1. N-Terminal protein sequence of the annexin family.
The N-terminal domain sequences of human annexin I - VI, VII (from residue 140 to 174), and VIII, drosophila annexin IX and X, mammalian annexin XI (from residue 180 to 209), and hydra annexin XII are shown. The sequences are aligned with the inclusion of gaps (marked by the dashed lines) to emphasize the similarity between the protein sequences. All sequences begin with Met-1 except annexin VII, which begins with Pro-140, annexin IX, which begins with Ala-1, and annexin XI, which begins with Ala-180. Boxes highlight conserved sequences between the proteins. Asterix indicates conserved position of a tyrosine residue that has been shown to be phosphorylated by the EGF receptor kinase and $pp60^{v\text{-}src}$ in annexin I and annexin II, respectively. ≈P indicates conserved position of a threonine or serine residue that has been shown to be phosphorylated by protein kinase C in annexin I, annexin II, and annexin IV. The sequences were taken from: [48] for annexin I, [49] for annexin II, [36] for annexin III, [50] for annexin IV, [51] for annexin V, [37] for annexin VI, [47] for annexin VII, [52] for annexin VIII, [26] for annexins IX and X, [27] for annexin XI, and [25] for annexin XII.

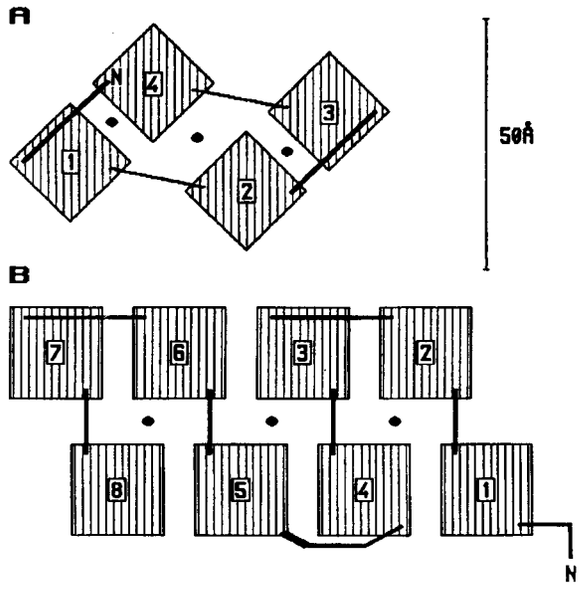

Fig. 2. Scheme of the domain arrangement and dimensions of annexin V (A) and annexin VI (B) derived from crystallographic data.
A, Repeats 1 and 4, and 2 and 3, respectively, form two tightly associated domains. Contact between them (in the center) is polar, thus generating a cation channel (from [2, 42], modified); B, Model of possible distribution of eight repeats (1 - 8) of annexin VI. The lines indicate the connecting segments (from [56], modified).

Table 4

Phosphorylation of annexins by protein kinases

EGFr, epidermal growth factor receptor protein tyrosine kinase; Ins r, insulin receptor protein tyrosine kinase; $pp60^{c\text{-}src}$, $pp60^{v\text{-}src}$, $pp50^{v\text{-}abl}$, retroviral protein tyrosine kinases; fps, protein tyrosine kinase; PKA, cAMP-dependent protein kinase; PTK, protein threonine kinase; PKC, protein kinase C; CamK, calmodulin-dependent protein kinase

Annexin		Protein kinase	Site of phosphorylation
I	monomer	EGFr, Ins r, $pp60^{c\text{-}src}$, $pp50^{v\text{-}abl}$ fps PKA PTK PKC	Tyr-21 Tyr-21 Tyr Thr-216 Thr Thr-24, Thr-41, Ser-27, Ser-28
II	monomer	$pp60^{v\text{-}src}$ Ins r, fps PKA, CamK PKC	Tyr-24 Tyr n.d. Ser-26
	heterotetramer	PKC	Ser-26(?)
III	monomer	PKC	n.d.
IV	monomer	PKC	Thr-9
V	monomer	EGFr, Ins r, $pp60^{src}$ PKC	n.d. n.d. Thr-22
VI	monomer	PKC (poorly)	n.d.
VIII		n.d.	Ser, Thr (?)
XII		hydra PKC-like kinase	n.d.

Data compiled from [1, 53, 54]; information about annexins VII, and IX - XI is not available; n.d. - not determined

two conflicting major opinions on the role of annexins within a cell [2 - 5]. On the one hand, it is postulated that different annexins perform different biological functions. On the other hand, it is possible that individual annexins are multifunctional and their function is being tissue-dependent (in analogy to calmodulin). It should be stressed, however, that both opinions are based mainly on the preferential cellular localization of some annexins in association with plasma membrane/cortical cytoskeleton and their ability to bind to phospholipids in a Ca^{2+}-dependent manner.

A suggestion that individual annexins may have a specialized role in cells and tissues came from the observations that different annexins exhibit unique tissue and cell distribution in vertebrates [4, 5]. Moreover, the cellular expression of various annexins during cell cycle [57], as well as during cell differentiation [5, 32, 58] and proliferation [5, 58] is different. Expression of annexins is also regulated by various hormones (e.g. the prolactin [1, 15], glucocorticoids [1, 2, 12, 15], and thyroid hormones [1, 15, 59]), and cell growth factors (e.g. the colony stimulating factor (CSF) [60], nerve growth factor (NGF) [5, 58], and epidermal growth factor (EGF) [58]).

There is a rapidly growing evidence that annexins may play a role in mediation of signal transduction [2, 61], control of cell proliferation [57, 58], and also in DNA synthesis, as components of the primer recognition protein (PRP) complex (PRP are cofactors for DNA polymerase alpha and may have a role in lagging-strand DNA replication) [34, 57, 62]. In regulation of membrane traffic annexins are believed to initiate membrane fusion in exo- and endocytosis [3, 17, 63 - 66], and to regulate cytoskeleton-membrane interactions (they bind F-actin and fodrin) [4, 5, 18, 19, 31, 67 - 69]. The participation of annexin II in aggregation of chromaffin granules and their fusion with plasma membrane during exocytosis after stimulation of chromaffin cells by acetylcholine is well documented. This protein establishes a link between secretory vesicles and membrane just before fusion [64], and this process requires probably phosphorylation of annexin II by protein kinase C [66].

In addition, it is postulated that annexins may play a role in calcium homeostasis [21, 22, 47, 70 - 72] (some of the annexins may form a voltage-dependent Ca^{2+} channel within a membrane [21, 22, 47, 70]), and act as enzymes [61]. As regulators of blood coagulation by binding to cell membrane annexins are thought to inhibit the association of blood coagulation factors with cell surface and to prevent their activation [1, 2, 5, 73]. In the case of anti-inflammatory action of glucocorticoids, annexins I-VI (lipocortins 1 through 6) are synthesized in response both to naturally occurring and synthetic glucocorticoids, inhibiting phospholipase A_2. This prevents the release of phospholipid arachidonic acid, the common precursor of both prostaglandin and leukotriene inflammatory mediators [1, 2, 13 - 15, 36].

The possible physiological role of members of the annexin family and some observations coming from experiments performed on reconstituted (artificial) models are listed in Table 5 and shown on Fig. 3.

Conclusions and perspectives

The family of annexin comprises at least eight different proteins, purified mostly from mammalian tissues (annexins I - VIII [1, 4, 52]), but also from *Torpedo marmorata* (annexins IV - VI [4, 18]), *Drosophila melanogaster* (annexins IX - X [26]), *Hydra vulgaris* (annexin XII [25]), *Dictyostelium discoideum* (annexin VII [29]), *Xenopus laevis* (annexin II [82]) and plant cells [28]. These proteins bind to natural and artificial membrane anionic phospholipids in the presence of calcium ions [4]. In all cases investigated to date, Ca^{2+} and phospholipids seem to act synergistically. This implies that calcium ions and lipid molecules might interact directly in the ternary complexes with annexins, as is believed to take place in the case of inhibition of phospholipase A_2 by lipocortins [6].

The rapid accumulation of structural and biochemical data should accelerate further progress toward defining the true activities of annexins. Since all vertebrate cells investigated to date express multiple forms of annexins, each potentially having a different cellular role, an effective investigation of annexin function may be facilitated by studying lower organisms that express fewer annexin gene products.

The discovery of annexins in lower eukaryotes created the opportunity for the use of genetic approaches to answer the question of the function of these proteins. With the use of the molecular recombination technique, three-

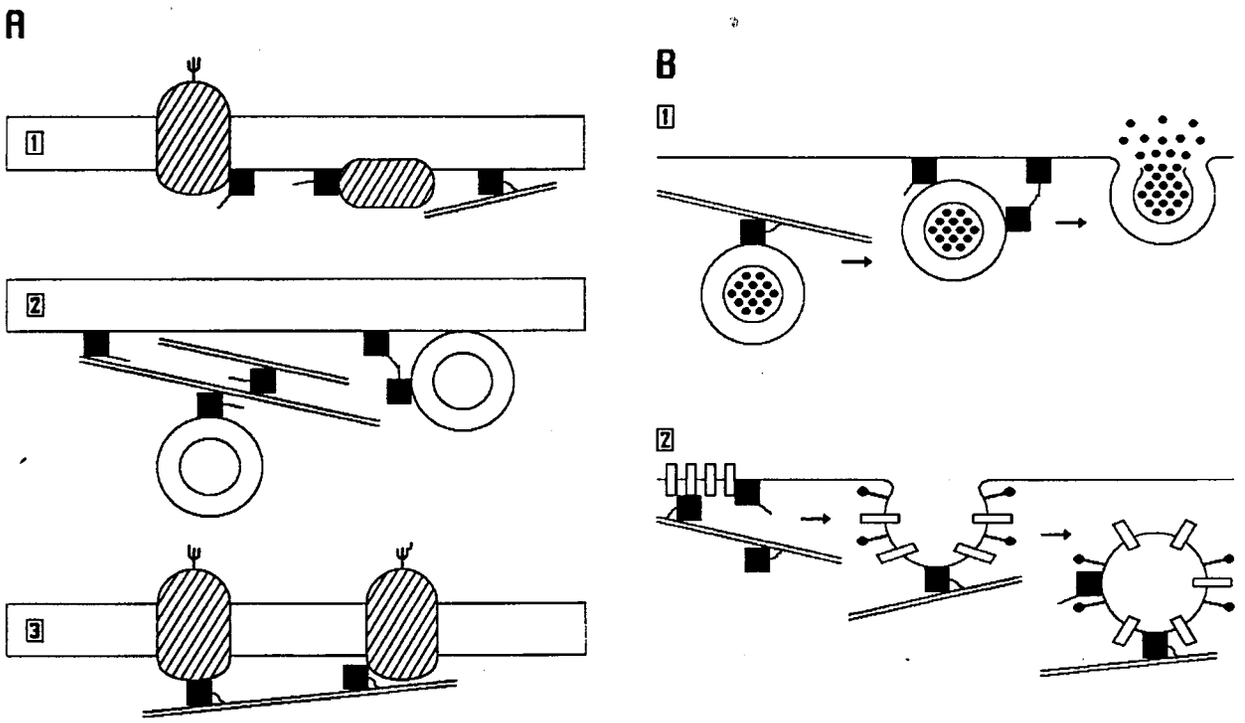

Fig. 3. Schematic diagram showing possible molecular interactions of annexins with other cellular components.

A, Interaction of annexins (squares) with plasma membrane and cytoskeletal elements (1); linking of secretory vesicle membranes to the plasma membrane by annexins (2); linkage of cytoskeletal elements to membrane receptors and crosslinking of membrane receptors (3). B, Possible interactions of annexins with membranes and cytoskeletal elements in exocytosis (1) and endocytosis (2). From [7], modified.

Table 5

Possible physiological function of annexins within a cell and in living organisms

Annexin	Possible biological function	Reference
I	**1.** participates in mitogenic signal transduction, cell growth and differentiation processes (human foreskin fibroblasts, rat PC-12 cells, adrenal pheochromocytoma cells);	[1, 2, 5, 58]
	2. regulates the phospholipase A_2 activity, playing a key role in the anti-inflammatory response to glucocorticoids;	[12 - 14]
	3. mediates cytoskeleton-membrane interactions (binds F-actin and fodrin; bundles actin filaments);	[1, 5, 18]
	4. substrate of tissue transglutaminase in A431 cells (human epidermoid carcinoma cell line);	[74]
	5. plays a role in sperm Ca^{2+}-mediated events;	[75]

II	**1.** regulates cell growth and cell transformation, by acting as coupling agent between growth factor receptors and their cellular targets;	[1, 4, 5]
	2. participates in cytoskeleton-membrane interactions (binds F-actin and fodrin; bundles actin filaments);	[12, 19, 31]
	3. inhibits phospholipase A_2, A_1 and lysophospholipase activities in porcine intestinal epithelium and also plays a crucial role in anti-inflammatory response to glucocorticoids;	[12, 14, 76]
	4. involved in controlling of secretion and cell motility;	[4]
	5. participates in Ca^{2+}-dependent exocytosis of chromaffin granules;	[16, 63, 64, 66]
	6. overexpressed (p36) in multidrug resistant small cell lung cancer (cell line H69AR);	[77]
	7. involved in DNA replication (subunit, together with 3-phosphoglycerate kinase, of primer recognition protein complex in HeLa and pancreatic carcinoma cells);	[34, 57, 62]
	8. participates in mitogenic signal transduction and cell proliferation;	[57]
	9. plays a role in blood coagulation;	[15]
III	**1.** an enzyme of the phosphatidylinositol signaling pathway (identical to 1-D-*myo*-inositol-1,2-cyclic-phosphate 2-inositolphosphohydrolase, EC 3.1.4.36);	[61]
	2. regulates blood coagulation;	[5, 15]
	3. stimulates cell growth;	[2]
IV	**1.** component of cytoskeleton in intestinal epithelium and rabbit enterocytes (binds F-actin);	[1, 32, 67]
	2. a potent mediator of exocytosis;	[17]
	3. reveals an anticoagulant activity;	[15]
	4. regulates phospholipase A_2 activity;	[1, 67]
	5. plays a role in phototransduction in crayfish *Orconectes limosus* (substrate for the protein carboxyl methyl transferase);	[78]
V	**1.** plays a regulatory role in blood coagulation;	[5, 15, 73]
	2. *in vitro* able to form voltage-gated calcium ion channel when reconstituted into liposomes, selective for divalent cations according to the following series: Ca>Ba>Sr>Mg;	[2, 22]
	3. inhibits protein kinase C-mediated phosphorylation of annexin I and myosin light kinase substrates;	[79]
	4. regulates various aspects of inflammation (inhibits phospholipase A_2) and immune response;	[4, 5]
	5. plays a role in the interaction of chondrocytes and fibroblasts with extracellular collagen and in cell differentiation;	[53]
	6. may be involved in the regulation of structural organization of membranes;	[33]
VI	**1.** most likely contributes to control membrane-microfilament interactions (binds F-actin);	[1, 68, 69]
	2. involved in exo- and endocytosis;	[16, 65, 68]
	3. promotes chromaffin granule fusion in adrenal medulla;	[17]
	4. may play a role during the contraction/relaxation cycle of skeletal muscles (modifies the Ca^{2+} gating of Ca^{2+} release channels in sarcoplasmic reticulum membranes);	[71, 72]
	5. regulates formation of coated vesicles within the cell (required for budding of clathrin-coated pits);	[65]
	6. probably plays a role in membrane traffick, and in the	

	regulation of structural organization of membranes;	[33, 65]
	7. is a calcium receptor protein of mitochondria;	[68]
	8. plays a role in initiation of DNA synthesis;	[80]
	9. participates in cell differentiation in mammary glands;	[81]
VII	1. promotes chromaffin granule fusion in adrenal medulla;	[3, 17, 70]
	2. in artificial and natural membranes acts as a calcium channel operating in a voltage-dependent manner;	[21, 22, 47]
VIII	1. affects blood coagulation;	[52]
	2. regulates phospholipase A_2 activity;	[52]

dimensional crystal structure analysis and the understanding of regulation of annexin gene expression the emergence of conclusive functional data in the near future is highly probable.

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