The Lipocortin/Calpactin Family of Calcium-Binding Proteins

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Evolutionary conservation and structural determinants of the calelectrins (annexins)

PAUL A. JOHNSTON and THOMAS C. SUDHOF
Department of Molecular Genetics and Howard Hughes Medical Institute, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235, U.S.A.

The calelectrins were originally characterized as a family of Ca\(^{2+}\)-dependent membrane-binding proteins [1–4] which were previously described as the Torpedo protein [5–7], because they exhibited similar biochemical properties and shared immunologic epitopes. Sequences from several of the calelectrins [8, 9] revealed them to be homologous to a number of other proteins that were independently characterized in recent years by several investigators. Members of this class of proteins can be considered as large multigene family that have been described under the following names: lipocortins [10], p35 and p36, also named calpactins I and II, and endonexins I and II, which were the 32.5 kDa and 35 kDa calelectrins [1, 2] renamed by Geisow et al. [8]; proteins I, II, and III [13]; lymphocyte Ca\(^{2+}\)-binding protein p68 [14]; chromobindins [15], and human placental anticoagulant protein [16]. All these proteins were isolated from vertebrates. Although no specific function has been demonstrated for these proteins which is accepted by all investigators, recently a consensus was reached to name all proteins from this superfamilly ‘annexins’.

The annexins are Ca\(^{2+}\)-binding proteins that bind to negatively charged phospholipids in a Ca\(^{2+}\)-dependent manner [1, 6, 17–20]. The amino acid sequences of all annexins predict the presence of four internal repeats with the 67 kDa calelectrin (annexin VI) containing a duplication of the four to eight such repeats [9, 14]. When the sequences of different members of the annexin family are compared with each other, they are most of the residues that are found to be conserved better by position between distinct members of the family than within a given protein [9]. The four repeats have differences in length that are conserved in all proteins with the third repeat being the longest (generally 85 residues), and the second the shortest (generally 72 residues). No sequences homologous to the EF-hand sequence of Ca\(^{2+}\)-binding proteins can be found in the primary structure of the annexins, suggesting that they contain a different Ca\(^{2+}\)-binding structure.

In addition to binding to negatively charged phospholipids in a Ca\(^{2+}\)-dependent manner, annexins I and II (lipocortins I and 2, also referred to as p35 and p36 or as calpactins I and 2 [21–23]) are tyrosine phosphorylated by growth-factor receptors in a stimulation-dependent manner [21–24]. The functional significance of the tyrosine phosphorylation is unknown, but the fact that annexins I and II are major substrates for the growth factor receptor tyrosine kinases indicates that they are intracellular proteins.

The different members of the annexin protein family are differentially distributed in vertebrate tissues, with some proteins showing very restricted localizations. For example, annexin IV (32.5 kDa calelectrin) is almost exclusively localized to ducal epithelia, such as those of biliary and pancreatic ducts [25], while annexin II (lipocortin 2/p36) is preferentially found in microvilli [26]. On the other hand, annexin VI (67 kDa calelectrin), appears to be ubiquitously found in all cells examined, although it is more concentrated in endocrine tissues [25].

Several, sometimes conflicting hypotheses have been proposed regarding the functions of the annexins. As lipocortins, they were thought to represent humoral mediators of the glucocorticoid-dependent regulation of inflammation [27]; as calpactins, to represent Ca\(^{2+}\)-dependent actin-binding proteins [23]; and as anticoagulants, to consist of circulating components of the coagulatoin system [16]. Recently, an annexin was suggested to be identical with inositol 1,2-cyclic phosphate 2-phosphohydrolase [28]. None of these proposed functions is universally accepted, since it is controversial whether the annexins are secreted or intracellular proteins, and because most members of the protein family do not bind actin [1]. We originally hypothesized that these proteins might be involved in regulating membrane traffic [1], and recent evidence has implicated annexin I (lipocortin I/calpactin) in regulating exocytosis in chromaffin cells [29]. However, a distinct cellular function for this protein family remains to be established.

Previously, the annexins have only been studied in vertebrates. Most recently, novel homologues of the annexins/calelectrins have been characterized in Drosophila melanogaster [30] and in Hydra [31]. Two novel annexins were identified by cDNA cloning from D. melanogaster. The two Drosophila annexins are 46% identical with each other, and most of the residues that are conserved better by position in the vertebrate annexins are also conserved in vertebrate annexins. A similarly high degree of homology relates Drosophila annexins to all different vertebrate annexins (40–50% identity). Together these sequence comparisons demonstrate that the Drosophila annexins are not the invertebrate homologues of particular mammalian annexins, but that they constitute novel members of the annexin gene family, and they were named annexins IX and X in continuation with a recently established terminology. RNA blots indicate that the messages for the two Drosophila proteins are differently expressed in development, with one message being expressed throughout development, whereas the other message is only found in early embryos and adult flies. In situ hybridizations localized the two Drosophila genes to 93B and 19A-4,7. Considering the fact that vertebrate organisms contain at least eight annexins, it seems likely that Drosophila contains additional members of this gene family. The two currently known Drosophila annexins [30] were cloned from an adult...
The biochemical properties of Drosophila annexin X were investigated using recombinant protein and compared with those of vertebrate annexins/calelectins. Similar to vertebrate annexins, annexin X bound to liver membranes and to liposomes containing phosphatidylserine in a Ca$^{2+}$-dependent manner, but not to liposomes containing phosphatidylcholine [30]. In addition, to test if annexin X expressed in other stages of the aqueous and detergent phases of Triton X-114 was investigated. Only in the presence of Ca$^{2+}$, but not of Mg$^{2+}$, did annexin X partition into the detergent phase of Triton X-114, supporting the notion that Ca$^{2+}$ introduces a conformational change in the protein that results in more hydrophobic characteristics. Together these data indicate that the Drosophila annexins, just like their vertebrate counterparts, interact with phospholipid bilayers in a manner that is dependent both on a negatively charged headgroup and on hydrophobic interactions.

The conservation of the annexin family of Ca$^{2+}$-binding proteins in invertebrates suggests that they have a basic function in cells which is not peculiar to vertebrate biology. The fact that all eukaryotic cells in multicellular organisms seem to express annexin genes will open avenues for mutational studies of these functions. What could these ubiquitous functions be? It has become apparent that both regulated and constitutive secretion are Ca$^{2+}$-dependent [32, 33]. Whereas the tissue distribution of the annexins/calelectins rules out a specific role related to regulated secretion, their properties are certainly consistent with a role in general membrane trafficking events.


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A review of studies leading towards a three-dimensional understanding of the annexin family of proteins

PAUL S. FREEMONT

Protein Structure Laboratory, Imperial Cancer Research Fund Laboratories, P.O. Box 125, Lincoln’s Inn Fields, London WC2A 3PX, U.K.

The annexin family of membrane-associated Ca$^{2+}$-binding proteins were first characterized by their ability to inhibit phospholipase A$_2$ and to bind to phospholipids in a Ca$^{2+}$-dependent manner [1]. A large body of biochemical data relating to different members of the family, has led to suggestions that the annexins may be involved in phospholipase A$_2$ and inflammatory regulation [2], membrane-membrane fusion [3], cytoskeletal organization [4], blood coagulation [5] and signal transduction [1]. However, the annexin family is widely distributed in nature, from animals to plants to insects, which suggests that the annexins have a general physiological role shared by all cell types and multicellular organisms [6]. In this short review, those studies aimed at discerning the three-dimensional structures of the annexin family of proteins will be discussed, including progress of our own work on the crystal structure determination of placental annexin IV.

Abbreviation used: ICaBP, intestinal Ca$^{2+}$-binding protein.