Review

Copines: a ubiquitous family of Ca$^{2+}$-dependent phospholipid-binding proteins

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Abstract. The copines are a novel family of ubiquitous Ca$^{2+}$-dependent, phospholipid-binding proteins. They contain two Ca$^{2+}$- and phospholipid-binding domains known as ‘C2 domains’ present in proteins such as protein kinase C, phospholipase C and synaptotagmin. Copines are thought to be involved in membrane-trafficking phenomena because of their phospholipid-binding properties. They may also be involved in protein-protein interactions since they contain a domain similar to the protein-binding ‘A domain’ of integrins. The biochemistry, gene structure, tissue distribution and possible biological roles of copines are discussed, including recent observations with Arabidopsis that indicate that copines may be involved in cell division and growth.

Key words. Copines; calcium; C2 domain; phospholipids; Arabidopsis; integrin A domain.

Discovery of copines

Copines are a family of proteins first identified in preparations of Ca$^{2+}$-dependent, phospholipid-binding proteins obtained from Paramecium tetraurelia [1]. These proteins are obtained by binding to phospholipid membranes in the presence of Ca$^{2+}$. Unlike similar preparations from other organisms, which consist mostly of annexins, the dominant species in Paramecium preparations is a single, 55-kDa band. Annexins are ubiquitous proteins thought to be involved in a host of Ca$^{2+}$-dependent cellular processes [2]. Early indications that the Paramecium protein was not an annexin or any other known protein were provided by the sequences of peptides produced by proteolytic digestion of the 55-kDa band. Further support for this observation was obtained by polymerase chain reaction (PCR) amplification of P. tetraurelia cDNA using primers designed according to the peptide sequences. Sequencing of the major amplification product – a 920-base pair product – showed that the 55-kDa protein contained two regions of strong similarity to the Ca$^{2+}$-dependent, phospholipid-binding domain known as C2, and a unique carboxy-terminal region or core domain that shows only a distant relationship to other proteins. This observation explained the biochemical properties of this protein and prompted the coinage of a name. Because of its behavior as a ‘companion’ of lipid membranes and the country where the initial observations were made (France), the feminine French noun ‘copine’ (pronounced ‘ko-peen’) was chosen. The feminine form was given because French nouns have genders and proteins are feminine.

Copines in nature

Proteins containing C2 domains exhibit Ca$^{2+}$-dependent binding to phospholipids. Some of them are enzymes such as protein kinase C (PKC) and phospholipase C (PLC), while others, such as synaptotagmin, rabphilin and Munc 13, are thought to play roles in exocytosis and membrane-trafficking phenomena [see refs 3 and 4 for reviews]. Data
base searches with the *Paramecium* sequence showed the existence of uncharacterized sequences similar to that of copine in a host of organisms such as green plants, nematodes and humans. Analysis of human cDNA sequences indicated that there were five different human copine genes which were referred to using roman numerals as human copines I–V [1]. Since then, two additional human copines have been reported: copine VI or N-copine [5] and copine VII [6]. The degrees of identities in the known regions of overlap between copine I, the most abundant form in the expressed sequence tag (EST) databases, and the other copines are 60, 78, 53 and 56% for copine II through V, respectively. Five genes have also been characterized in *Caenorhabditis elegans*. Comparison of human copine with copines from other organisms in their region of overlap reveal 40, 40 and 33% identities for *C. elegans*, *Arabidopsis* and *Paramecium*. Further experimental studies of *Paramecium* cDNA showed the presence of two closely related genes for copine (CPN1 and CPN2) that are both expressed in wild-type *Paramecium*. Regarding expression of copines in humans, a quite comprehensive screen was recently carried out [7] using cDNA probes for copines I through VI to probe a 76 human tissue-type MTE array (Clontech). Copines I, II and III are expressed ubiquitously. Copine IV expression is restricted to brain, heart and prostate, whereas copine VI was brain specific as previously reported [5]. The actual content of copine proteins in human tissues has not been systematically investigated. We have carried out, however, a screening of different mouse and bovine tissues using antico- pine I antibodies and found that copine I is ubiquitously expressed as detailed below. Figure 1A shows the alignment of all seven human copine sequences obtained using data from current databases. A dendrogram illustrating the relationships between these sequences is presented in figure 1B.

Figure 1A. Sequence alignment of the human copines. (A) The predicted amino acid sequences of the seven human copines were aligned using the PRETTY program of the Genetics Computer Group Wisconsin Package. The Consensus sequence represents residues that appear in the same position in at least four of the seven copines. Asterisks mark the positions of residues conserved in all seven copines. The positions of the C2 and A domains are marked by underlining. The residues in the C2 domains that are thought to coordinate calcium are marked with a \(C\). The residues in the A domain that are thought to coordinate magnesium are marked with an \(M\). The sequences for copines I, III, VI and VII were obtained from current GenBank entries for full-length proteins. The sequences for copines II, IV and V were inferred from current GenBank EST entries.