A new group of Ca\(^{2+}\)-binding proteins which reversibly associate with biomembranes has been identified in many different cell and tissue types (reviewed in Owens & Crump-ton, 1984). A characteristic common property of these proteins is an ability to bind at micromolar Ca\(^{2+}\)-concentrations to bilayers composed of acidic phospholipids normally present at the cytoplasmic face of biomembranes. A further feature of these molecules is their ability to potentiate the aggregation of membrane vesicles at increased concentrations of Ca\(^{2+}\). The basic structural feature of this new Ca\(^{2+}\)-regulated protein family is a homologous amino acid domain of 70–72 amino acids, which is repeated four or more times. Subtle variations of this core domain structure and rather short regions of reduced sequence homology are associated with different levels of expression in cells, variations in subunit structure, affinity for cytoskeletal proteins or substrate specificity for cellular kinases. This article examines the cell and tissue distribution of members of this new protein family and proposes a model for their mode of interaction with biomembranes.

Abbreviations used: CDMBP, Ca\(^{2+}\)-dependent membrane-binding protein; ICaBP, intestinal calcium-binding protein; PE, phosphatidylethanolamine.

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### Table 1. Nomenclature of Ca\(^{2+}\)-dependent membrane-binding proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Mol. mass (kDa)</th>
<th>pI</th>
<th>Other names</th>
</tr>
</thead>
<tbody>
<tr>
<td>p70</td>
<td>68–70</td>
<td>5.9</td>
<td>Lymphocyte p68, calcemedin (67 kDa), Protein III (liver), chromobindin 20, Synhibit</td>
</tr>
<tr>
<td>p36</td>
<td>36</td>
<td>7.5</td>
<td>p36 (p39, p34), calpactin I, lipocortin II, protein I (intestine), lymphocyte 33, chromobindin 8</td>
</tr>
<tr>
<td>p35</td>
<td>35</td>
<td>6.8</td>
<td>Calpactin II, lipocortin I, lipomodulin, chromobindin 9</td>
</tr>
<tr>
<td>Calelectrin</td>
<td>34–36</td>
<td>5.5–6.0</td>
<td>–</td>
</tr>
<tr>
<td>p32.5</td>
<td>32.5</td>
<td>5.6</td>
<td>Endonexin, protein II (intestine), lymphocyte p28</td>
</tr>
</tbody>
</table>

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### Localization and structure of novel calcium-regulated phospholipid-binding proteins

A new group of Ca\(^{2+}\)-binding proteins has been identified in many different cell and tissue types (reviewed in Owens & Crump-ton, 1984). The characteristic common property of these proteins is an ability to bind at micromolar Ca\(^{2+}\)-concentrations to bilayers composed of acidic phospholipids normally present at the cytoplasmic face of biomembranes. A further feature of these molecules is their ability to potentiate the aggregation of membrane vesicles at increased concentrations of Ca\(^{2+}\). The basic structural feature of this new Ca\(^{2+}\)-regulated protein family is a homologous amino acid domain of 70–72 amino acids, which is repeated four or more times. Subtle variations of this core domain structure and rather short regions of reduced sequence homology are associated with different levels of expression in cells, variations in subunit structure, affinity for cytoskeletal proteins or substrate specificity for cellular kinases. This article examines the cell and tissue distribution of members of this new protein family and proposes a model for their mode of interaction with biomembranes.

Abbreviations used: CDMBP, Ca\(^{2+}\)-dependent membrane-binding protein; ICaBP, intestinal calcium-binding protein; PE, phosphatidylethanolamine.

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### Subcellular fractionation

Ca\(^{2+}\)-dependent membrane-binding proteins (CDMBPs) were isolated from bovine adrenal medulla by homogenizing this tissue in the presence of 5 mM-EGTA, obtaining a post-microsomal supernatant and re-adjusting the free Ca\(^{2+}\) to a concentration of ~100 μM–1 mM. Small microsomal vesicles could be sedimented following Ca\(^{2+}\)-addition and after washing and re-extraction with 5 mM-EGTA, yielded the polypeptides listed in Table 1, together with more minor proteins which have not been further characterized. The identification of the adenral proteins separated from the EGTA eluate has been made by reference to their migration on two-dimensional gels (Geisow et al., 1984) and by Western blotting using specific antisera for the proteins described in the Table.

This technique of Ca\(^{2+}\)-dependent absorption and elution from membrane preparations has been used with minor variations to examine Ca\(^{2+}\)-dependent membrane-binding proteins in bovine liver and lung, rat gut, human placenta and electric organ tissue from the electric fish, *Torpedo marmorata*. In each of these tissues immunohistochemistry has localized the proteins within the component structures and cells.

In the absence of Ca\(^{2+}\) ions, the protein calelectrin is a major soluble protein of the electric organ of *Torpedo* or synaptosomal preparations made from the tissue. In contrast to the mammalian tissues examined, only one major Ca\(^{2+}\)-dependent membrane-binding protein was found although there is evidence for heterogeneity of calelectrin from isoelectric focusing, which may reflect sequence variants.
Immunofluorescence studies on the *Torpedo* electromotor system showed immunoreactive protein to be present throughout the electrocyte, axons and nerve terminals. Electron-microscopic immunolocalization indicated that calelectrin was enriched on the cytoplasmic surface of the acetylcholine receptor-containing membranes. The enrichment of all these proteins at the apical surface of the syncytiotrophoblast; p70 in particular was enriched in the microvilli.

In all tissues studied to date, different proportions of these proteins were isolated depending on the presence or absence of non-ionic detergent during extraction — p35, p36 and p70 required detergent for maximal recovery.

Despite the evident diversity of both type and level of expression of these proteins, several common factors emerge as a result of the subcellular localization studies. First, despite the expectation of low resting cell Ca\(^{2+}\) levels, all these proteins show a definite localization at the cell surface membrane or membrane-associated organelles. Secondly, the distribution of these proteins suggests that they have a role at membrane specializations, being enriched at brush-border membranes and in (*Torpedo*) synaptic membranes.

The publication of complete amino acid sequences for p35 and p36 (Wallner et al., 1986; Huang et al., 1986; Saris et al., 1986) and partial amino acid sequences for calelectrin and endonexin (Geisow et al., 1986) has explained the underlying similarity in functions of these proteins. Immunohistochemistry revealed the enrichment of all these proteins at the apical surface of the syncytiotrophoblast; p70 in particular was enriched in the microvilli.

![Sequence alignments of ICaBP with p35, p36 and endonexin (p32) emphasizing the pseudo-twofold repeat within the major repeat](image-url)

**Fig. 1.** Sequence alignments of ICaBP with p35, p36 and endonexin (p32) emphasizing the pseudo-twofold repeat within the major repeat.

Sequences p35 (blocks A: residues 46–346; Wallner et al., 1986) and p36 (blocks B: residues 35–339; Huang et al., 1986) are continuous and read alternately in the upper and lower blocks until absence of a continuation arrow (>) marks the C-terminus. The endonexin sequence (C) was established by overlapping tryptic CNBr and *Staphylococcus aureus*-V8 peptides sequences as previously described (Geisow et al., 1986). The boxes in D represent the joint secondary structure prediction (see text and Table 1 for details) of α-helix for A and B. Line E is the aligned sequence of bovine ICaBP and the boxes in F indicate the α-helix in its refined structure (Szébenyi et al., 1986). Consensus amino acid characteristics are indicated in the corresponding helical regions of F and D as; hydrophobic = ◦, polar = ○, and amphipathic = □. Proline residues (which often delimit α-helices) are boxed. The third α-helix of ICaBP is shaded as it is irregular.
proteins. Each molecule contains 70-72 amino acid repeats which have further twofold internal pseudosymmetry (Fig. 1). Structural considerations dictate that these repeats have a common fold and probably represent structural domains. Interestingly, there are no sequences which represent the E-F hand motif of most high-affinity Ca"^2^+"-binding proteins (Tuffy & Kreisinger, 1975). Because of the sequence redundancy and the known high z-helix content of these proteins (Geisow, 1986) we have attempted to predict the fold of a single structural domain. In Fig. 1, row D, the predicted location of z-helix is indicated by boxes (all the sequences were used to make this prediction). The emergence of a strong helix-loop-helix linking helix-helix-loop helix pattern (a characteristic of the parvalbumin, calmodulin family) prompted us to compare this predicted secondary structure with that of intestinal calcium-binding protein (ICaBP), whose X-ray structure is known at high resolution (Szebenyi & Moffat, 1986).

A striking correspondence of the hydrophobic residues between the helices of ICaBP and those predicted for the CDMBP's could be made by the acceptance of a major deletion in both Ca"^2^+"-binding loops of the ICaBP structure. Despite this deletion, both the conventional Ca"^2^+"-binding loops of ICaBP and the shortened versions predicted for the CDMBP domains each contain invariant glycine residues and conserved acidic groups.

Using an interactive model building program FRODO (Jones, 1978), the amino acids of the second repeat of p35 (row 2 in Fig. 1) were substituted for the corresponding residues of ICaBP (1ICB) whose atomic coordinates were obtained from the Protein Structure Data Bank (Bernstein 1977). The non-homologous regions between the two helices were modelled by making the most economic connections which did not alter the relative conformations of the four-helical bundle of ICaBP. The resulting structure was energy-refined to minimize steric clashes.

Analysis of the resulting model indicated that it had a hydrophobic core comprised of the conserved residues. One of the two Ca"^2^+" ions of the original ICaBP structure was well co-ordinated by two conserved acidic residues and main chain carbonyl oxygens, which had substantially re-oriented to provide an octahedral co-ordination sphere for the Ca"^2^+" ion. This co-ordination of Ca"^2^+" is not without precedent, since despite the presence of a conventional E-F hand the second Ca"^2^+"-binding loop of ICaBP uses mainly main chain carbonyl oxygens rather than alternate oxygen atoms contributed by side-chains. The connection between the fourth and fifth z-helices in the model was too short to bind Ca"^2^+" and this ion was deleted from the model.

The model for the structural domain differs substantially from the ICaBP structure in the region of the loops, giving the appearance of a deep cleft in the domain surface with a relatively accessible Ca"^2^+" ion at one end. This suggested a simple mode of interaction with phospholipid bilayers whereby the head group of an acidic phospholipid could be accommodated in the cleft with the phosphate replacing one or more of the ligands in the co-ordination sphere of bound Ca"^2^+".

'Docking' experiments using the known bilayer conformation of phosphatidylethanolamine (PE) (Allen et al., 1979) proved that this type of ternary complex is perfectly feasible. Indeed, it rationalizes the absolute conservation of the glycine residue in the loop, since no other residue type would permit the phosphate group to approach the Ca"^2^+" ion. The ionized amino group of PE makes ionic interactions with conserved acidic residues at the second (non-Ca"^2^+" binding) loop. (Phosphatidylycerine would be accommodated in a very similar fashion.) Experimental support for this model comes from the synergistic binding of Ca"^2^+" and phospholipids. In the presence of phosphatidylycerine or other acidic phospholipids, the affinity for Ca"^2^+" is increased by two to three orders of magnitude. This must occur either by the formation of a direct ternary complex as modelled or by an indirect allosteric effect of lipid upon the protein fold. The present model provides a working hypothesis for the interaction of members of the new protein family with membranes. Since each protein contains multiple domains, both binding and aggregation of membrane vesicles would be predicted. Work is now in progress to determine further experimental support for this model and to locate potential sites of interaction of p36, p35 and p70 with F-actin.

Huang, K. S. et al. (1986) Cell 46, 191-199
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The structure of desmosomes and their role in malignant disease

D. R. GARROD, E. P. PARRISH and J. E. MARSTON
C.R.C. Medical Oncology Unit and Department of Surgery, Southampton General Hospital, Southampton, Hants SO9 4XY, U.K.

Desmosomes are adhesive intercellular junctions of epithelial cells that link the intermediate filament cytoskeletons of adjacent cells. In epidermis, they consist of eight major constituents, which we shall designate dp (desmosomal proteins) and dg (desmosomal glycoproteins) (Miller et al., 1987). In descending order of relative molecular mass (M,) the proteins are dp1 (250,000), dp2 (23,000), dp3 (83,000) and dp4 (75,000), and the glycoproteins dg1 (175,000-164,000), dg2 (130,000), dg3 (115,000) and dg4 (22,000) (Skerrow & Matoltsy, 1974; Gorbsky & Steinberg, 1981; Franke et al., 1982; Cowin & Garrod, 1983; Garrod, 1985, 1986a; b; Miller et al., 1987). In addition to these are desmocamin, a high molecular mass calmodulin- and cytokeratin-binding protein (Tsukita & Tsukita, 1985), and a glycoprotein of 140,000 M, (Jones et al., 1986). Desmosomal proteins and 2 are biochemically and immunologically related to each other, as are dg2 and dg3, while the remaining molecules are distinct (Cohen et al., 1983; Mueller & Franke, 1983; Kapprell et al., 1985).