Cell biology

Consensus in exocytosis

from Robert H. Kretsinger and Carl E. Creutz

DURING exocytosis, the process by which eukaryotic cells release packets of molecules to the extracellular environment, the exocytotic vesicle buds off from the Golgi apparatus and fuses with the plasma membrane, releasing its contents at the time of secretion. Douglas[1] and Rubin[2] proposed that calcium couples cell stimulation to secretion, analogous to the coupling of excitation to contraction in muscle. Understanding these complex processes has been one of the more rewarding themes of cell biology during the past two decades. On page 636 of this issue[3] Geisow and colleagues report the homology of the protein p36 with calelectrin and the closely related proteins pII and endonexin, representatives of a family that may be involved in the coupling events in secretory cells.

There are many soluble proteins that bind to purified secretory vesicles or to other membranes and components of the cytoskeleton in a calcium-dependent manner *in vitro*[4,5]. Identification of isoforms and modified forms of these proteins is not complete. Calelectrin, a soluble calcium-binding protein isolated from the electroplax tissue of the ray *Torpedo marmorata* (a tissue very rich in cholinergic synaptic membranes), has a relative molecular mass (M_r) of 34,000. Endonexin (M_r = 32,500), another calcium-binding protein, has been identified in the adrenal medulla and in the liver; and the protein pII ($M_r \simeq 32{,}000$) has been identified in the brush border of the intestinal epithelium. But calelectrin, endonexin and pII could be the same protein; antibodies raised to calelectrin have been shown to react with endonexin and, in turn, antibodies to endonexin react with pII. The anti-calelectrin antibodies also react with p70 (M_r = 70,000) from liver and with p36 ($M_r \simeq 36{,}000$) from adrenal medulla and brush border.

This cross-reactivity indicates that the five proteins are similar and probably homologous. The new experiments of Geisow *et al.*[3] are straightforward and the results confirm these expectations. The authors cut calelectrin, endonexin, pII and p36 into fragments with trypsin or cyanogen bromide and purified the resulting peptides by chromatography. Several peptides react with antiserum to calelectrin, and Geisow *et al.* sequenced these and several others. Nine different, non-overlapping peptides — one each from p36 and from pII, two from calelectrin and five from endonexin — have similar amino-acid sequences, as summarized in the consensus sequence of the first line of the figure. The carboxy-terminal half of this 17-residue consensus sequence probably forms an α-helix; the first half is predicted to be non-helical.

When preparing this article, we noticed that the sequence of lipocortin (lipomodulin) recently published by Wallner *et al.*[6] contains three regions homologous with the endonexin fold that is defined by the consensus sequence. (The results of Wallner *et al.* were not published when Geisow *et al.* submitted their manuscript but they have noted them in proof.) Lipocortin exerts its anti-inflammatory effect by inhibiting phospholipase A_2, which would otherwise release arachidonic acid from the 2′ position of phospholipids. Arachidonate, in turn, is the precursor of leukotrienes and prostaglandins, inflammatory agents.

What is one to make of this amino-acid sequence information? The endonexin fold is not strongly hydrophobic; none of the homologues are inferred to be deeply embedded in a phospholipid bilayer, as are integral membrane proteins. The fold is not homologous with the EF-hand[7] of calcium regulatory proteins such as calmodulin, troponin C, S-100 and parvalbumin. Lipocortin also appears not to contain an EF-hand. In the EF-hand as well as in extracellular calcium-binding proteins such as thermolysin, the residues coordinating calcium are not adjacent in sequence. The loop of the endonexin fold contains three or four oxygen-containing side chains, depending on which of the nine domains described by Geisow *et al.*[3] is considered; however, the Thr-Asp-Glu residues of the consensus sequence are adjacent. One of the domains in lipocortin has only one oxygen ligand, and arginine and histidine are substituted for aspartate and glutamate in this loop. It will be very interesting to know the complete amino-acid sequence of the endonexin/p36 group of proteins and of synexin, which appears to have activities similar to those of endonexin. If these proteins do bind calcium under physiological conditions, as now seems likely, and if, like lipocortin, they lack EF-hands, they would be the first exceptions to the generalization that calcium-modulated proteins contain EF-hands.

A further interesting development in the calcium modulation story has recently been published. The protein p36, which apparently interacts with chromaffin granules and with components of the cytoskeleton in a calcium-dependent manner, can form a heterotetramer ($p36_2$, $p10_2$). The amino-acid sequence of p10 (ref. 8) has two EF-hands. Although in both domains the loops that might bind calcium differ from the archetype, p10 may still impart the calcium sensitivity to the heterotetramer. Perhaps again, as occurred with aqueorin and with calpain, the EF-hand generalization of calcium-binding proteins will be rescued when the complete amino-acid sequences of the other proteins are determined.

But as so often happens, a nice calcium story is sullied by the complication of phosphorylation. One specific tyrosine of p36 (residue 23) is phosphorylated by the tyrosine-specific kinase $p60^{v\text{-}src}$ (encoded by Rous sarcoma virus) and by similar kinases from other transforming viruses[9]. The cellular homologue of $p60^{v\text{-}src}$ is a component of the chromaffin-granule membrane[10] and therefore might phosphorylate p36 during exocytosis. Lipocortin, now known to be a homologue of p36, is also a substrate for a tyrosine-specific kinase. In murine thymocytes, treatment with a mitogen such as concanavalin A, the calcium ionophore A23187 or 4β-phorbol 12-myristate 13-acetate, leads to a marked increase in the tyrosine phosphorylation of lipocortin. In its phosphorylated form it no longer inhibits phospholipase A_2 and more arachidonic acid is released. Wallner *et al.*[6] identified Tyr-21 as the probable phosphorylation site of lipocortin; the first endonexin fold begins at Met-56. Will the Tyr-23 of p36 also be 35 residues upstream of its endonexin fold? □

		Lys-Gly-fob-Gly-Thr-Asp-Glu-var-var-Leu-Ile-fil-Ile-Leu-Ala-fil-Arg
Lipocortin residues	56-72	Met-Val-Lys-Gly-Val-Asp-Glu-Ala-Thr-Ile-Asp-Ile-Leu-Thr-Lys-Arg
Lipocortin residues	128-144	Lys-Gly-Leu-Gly-Thr-Asp-Glu-Asp-Thr-Leu-Ile-Glu-Ile-Leu-Ala-Ser-Arg
Lipocortin residues	287-303	Lys-Gly-Val-Gly-Thr-Arg-His-Lys-Ala-Leu-Ile-Arg-Met-Val-Ser-Arg

Consensus amino-acid sequences found in endonexin, calelectrin, pII and p36 compared with three homologous domains in lipocortin. Carboxy-termini to the right. Abbreviations: fob, hydrophobic; fil, hydrophilic; var, variable.

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