Cell biology

Calpactin in exocytosis?

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THE exocytotic fusion of membrane vesicles with the plasma membrane probably occurs in all cells and is involved, for example, in the insertion of new components into the plasma membrane and in the release of secretory products from vesicles. In the case of cells specialized to secrete in a regulated fashion, exocytosis is triggered on cell activation by an intracellular signal which is often a rise in the concentration of cytosolic calcium. The importance of calcium in the regulation of exocytosis has led to a search for putative calcium receptors involved in mediating the interaction and fusion of secretory vesicles with the plasma membrane. A report by Drust and Creutz on page 88 of this issue¹ points to a possible role for the protein calpactin in the calcium-dependent control of membrane fusion.

In attempts to identify calcium-regulated proteins that could be involved in exocytosis, several proteins were identified in adrenal medullary extracts that could be isolated because of their ability to bind reversibly to secretory vesicle membranes in the presence of micromolar concentrations of calcium^{2,3}. Among these proteins are calmodulin, protein kinase C (ref. 4), caldesmon⁵ and a series of closely related calcium- and lipid-binding proteins, now known to be widely distributed, which have been given the generic name annexins⁶. Earlier data from protein and complementary DNA sequence analysis had shown significant similarity between some of the annexins⁷, and further recent results⁸⁻¹⁰ show that there are at least five separate members of this family (calpactin, lipocortin I, endonexin, endonexin II and p70) which share conserved domains. Calpactin differs from other annexins in being a heterotetramer with the structure p36₂ p10₂. The heavy chain of calpactin (p36) was already known as a major substrate for phosphorylation by retroviral tyrosine kinases.

One test of the possible significance of calcium-dependent binding of annexins to membranes is whether they bind at physiologically relevant calcium levels. The levels of calcium required for binding of annexins to membranes or liposomes are variable⁷ (see table) but it is likely that all these proteins become associated to some extent with membranes following cell activation and elevation of calcium to low micromolar levels. Drust and Creutz¹ address the additional question of whether any of the annexins mediate membrane-membrane interactions in stimulated cells. Their data (see ref. 13 for data on p70) show that only calpactin is able

to aggregate secretory granules at low micromolar levels of calcium, and that the calcium-dependency is similar to that of calcium-activated secretion in permeabilized chromaffin cells. Truncated forms of the calpactin heavy chain, which are unable to bind p10, show much higher calcium requirements for granule aggregation¹. Powell and Glenney¹¹ have also recently found that intact calpactin will aggregate liposomes at lower calcium levels than the isolated heavy chain. These results suggest that the ability of calpactin to aggregate secretory granules or liposomes at much lower calcium levels than the other annexins may be conferred by

use in finally determining the importance of calpactin in the permeabilized cell. Chromaffin cells permeabilized by treatment with digitonin will secrete in response to addition of micromolar levels of calcium. If after permeabilization, however, the cells are maintained at low calcium levels, they eventually leak proteins essential for exocytosis, and secretory responsiveness can be reconstituted by adding back the leaked proteins¹⁵. The factor(s) lost from the cells cannot be replaced by re-addition of calmodulin, nor can loss of secretory responsiveness be prevented by treating the cells with phorbol ester to reduce leakage of protein kinase C. The conditions used for incubation of the cells at low calcium are such that it is likely that calpactin and other annexins would be among the leaked proteins. An examination of the effect of calpactin on cells that have become refractory to calcium stimulation because

Calcium-dependence of annexin binding to membranes or liposomes

Protein	Concentration of Ca ²⁺ for half-maximal binding (μM)	Ref.
p70*	2.6	2
lipocortin [†]	4 [§] , 20	17
endonexin I (32K calelectrin)*	5.5	16
endonexin II [‡]	40	9
calpactin [†]	<0.01 , 5	11

The table lists the five distinct proteins that have been shown to be members of the annexin family on the basis of their possession of conserved common domains. For alternative names that have been given to these proteins, see ref. 6.

*Binding assayed with chromaffin granule membranes.

[†]Binding assayed with phosphatidylserine liposomes.

[‡]Binding assayed with phosphatidylethanolamine liposomes.

[§]Data for phosphorylated form.

^{||}A large component of binding was detected at low calcium levels, with a second calcium-dependent component.

p10. The association of p10 could increase the affinity of the calcium and/or lipid-binding sites within the heavy chain or, alternatively, the heterotetrameric structure of intact calpactin could facilitate membrane crosslinking.

Is calpactin the calcium receptor in exocytosis? The results of Drust and Creutz show that at least in an *in vitro* model of membrane interaction calpactin can mediate membrane-membrane association, which in the presence of Mg²⁺ and arachidonic acid results in membrane fusion. There is as yet no direct evidence that such a mechanism occurs during exocytosis. Calpactin is localized in the subplasmalemmal region of chromaffin cells¹² and shows increased incorporation of radiolabelled phosphate on stimulation¹³, which could indicate a phosphorylation-dependent mechanism for inactivation of calpactin¹¹ following exocytosis. Also arachidonic acid is produced on stimulation in chromaffin cells¹⁴, so it is possible that exocytotic fusion in these cells involves the same components that are effective in the *in vitro* experiments.

An experimental model likely to be of

of protein leakage may well help to determine whether this protein is indeed involved in exocytosis. □

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