synthesized sucrase-isomaltase with the basolateral membrane fraction may have been due to a co-purification of basolateral membranes with transport vesicles for which no marker proteins are known.

The differentiated colon carcinoma cell line Caco 2 represents a promising cell culture model for studying molecular mechanisms underlying intracellular membrane traffic and the biogenesis of surface membrane polarity in epithelial cells. The use of a cell culture system which closely mimicks the epithelial organization *in vivo* has a number of advantages in comparison with whole tissue as the experimental system. In particular, a cell culture system provides a homogeneous cell population and allows easy experimental manipulation. Although more difficult with cell cultures, subcellular fractionation of Caco 2 cells is feasible and can yield membrane fractions whose purity approaches that obtained with whole tissue. Progress in our understanding of the biosynthetic pathway of surface membrane proteins has mainly come from studies on virus maturation in Madin–Darby canine kidney cells (Simons & Fuller, 1985). It will now be possible to investigate these mechanisms with endogenous membrane proteins in Caco 2 cells.

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Phosphoprotein B-50 and phosphoinositides in brain synaptic plasma membranes: a possible feedback relationship

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In the last decade research on the role of phosphoproteins in neuronal function has yielded evidence that suggests a role for certain phosphoproteins in receptor-activation, receptor-mediated transmembrane signal transduction, ion conductance, etc. At first, attention was focused on cyclic AMP-sensitive protein kinases and their substrates leading to the identification of a neuron-specific presynaptic protein called synapsin I (Rodnight, 1982; Nestler & Greengard, 1983). In parallel, interest was directed at Ca^{2+}/calmodulin and Ca^{2+}/lipid-sensitive kinases and their substrates present in the synaptic region (Rodnight, 1983). One such line of research led to the identification and characterization of the synaptic protein B-50 (M_r 48000, pH 4.5; Zwiers *et al.*, 1980*a*). It is likely that the B-50 protein is identical to protein band $\gamma 5$ (Gower & Rodnight, 1982), F_1 (Routtenberg, 1982), 47 K (Hershkowitz *et al.*, 1982) or P45p(Ca) (Mahler *et al.*, 1982). In this paper, our present understanding of the role of this protein in synaptic membrane function is reviewed.

Localization of B-50 protein

Using two-dimensional separation techniques and anti-B-50 antisera, the B-50 protein could be detected only in the particulate fractions of brain homogenate and not in subcellular fractions of other rat tissues studied (Kristjansson *et al.*, 1982). Immunostaining of B-50-like proteins in brain homogenates of various vertebrate species revealed the presence of B-50 in human, rat, mouse, hamster, rabbit, cow and chick brain. No B-50 immunoreactivity was obtained in homogenates from *Xenopus*, goldfish and trout brain (Oestreicher *et al.*, 1984).

Although endogenous B-50-phosphorylating activity was detected throughout the rat brain, a clear regional activity was obtained. The order of decreasing activity was septum > hippocampus and neocortex > thalamus > cerebellum > medulla oblongata > spinal cord (Kristjansson *et al.*, 1982). Recently, a radioimmunoassay for the B-50 protein has been developed using phosphorylated B-50 as tracer and affinity-purified anti-B-50 immunoglobulins. It was found that synaptic plasma membranes from total rat brain contained 10 μg of B-50/mg of protein and regional studies confirmed the pattern previously reported for the endogenous B-50 phosphorylation activity in brain (septum > hippocampus or cortex $\gg$ cerebellum; Oestreicher *et al.*, 1983, 1985).

Immunohistochemical studies of various rat brain areas at the light microscopic level revealed dense immunostaining of B-50 in regions rich in synaptic contacts, whereas white matter and cell perikarya were virtually unstained (Oestreicher *et al.*, 1981). Recently, we studied the ultrastructural localization of B-50 in ultrathin cryosections of fixed hippocampal tissue and synaptosomes by means of highly specific anti-B-50 immunoglobulins and the protein A–gold staining procedure. Immunoreactivity for B-50 was present exclusively at presynaptic sites of nerve terminals *in situ*, presumably in synaptic vesicles and closely associated with the inner surface of the presynaptic plasma membrane. Not all synaptosomes stained positively for B-50, pointing to a possible association with some, rather than all, transmitter systems (Gipsen *et al.*, 1985*a*). Using subcellular fractionation techniques followed by endogenous

Abbreviations used: PtdIns4*P*, phosphatidylinositol 4-phosphate; PtdIns4,5P_2, phosphatidylinositol 4,5-bisphosphate; ACTH, adrenocorticotropic hormone; pB-50, phospho-B-50; DG, diacylglycerol, InsP_3, inositol trisphosphate.

phosphorylation of B-50, Sörensen *et al.* (1981) also concluded that the B-50/B-50 kinase complex is associated with the presynaptic membrane.

B-50 and the activity of phosphatidylinositol 4-phosphate kinase

The endogenous B-50-phosphorylating activity was solubilized from the synaptic plasma membranes and enriched by ion-exchange chromatography and ammonium sulphate precipitation. In the precipitate formed between 55 and 80% saturation (ASP_{55-80}) endogenous phosphorylation using [γ-^{32}P]ATP labelled only the B-50 protein (Zwiers *et al.*, 1980*a*). Subsequent studies demonstrated the presence of proteases as well as phosphatidylinositol 4-phosphate (PtdIns4*P*) kinase activity (Jolles *et al.*, 1980; Zwiers *et al.*, 1980*b*). In a number of chromatographic steps the B-50 protein kinase/B-50/lipid kinase activity were inseparable; consequently it was assumed that they were part of a multifunctional enzyme complex.

Studies on the significance of such a complex revealed that the degree of phosphorylation of B-50 in the ASP_{55-80} fraction, as well as in a lysed crude mitochondrial/synaptosomal fraction, were inversely related to the conversion of exogenously added PtdIns4*P* to phosphatidylinositol 4,5-bisphosphate (PtdIns4,5P_2) (Jolles *et al.*, 1980, 1981). A variety of indirect evidence has furnished support for this reciprocal relationship. The extent of PtdIns4,5P_2 formation was influenced by prephosphorylation of B-50 for various times, specific inhibition of the B-50 phosphorylation by adrenocorticotropic hormone $(ACTH)_{1-24}$ and anti-B-50 immunoglobulins, and transmitter-receptor activation followed by *post hoc* phosphorylation of B-50 and the polyphosphoinositide (Jolles *et al.*, 1980; Oestreicher *et al.*, 1983; Jork *et al.*, 1984). The findings of other authors have also suggested an inverse relationship between protein phosphorylation and the labelling of phosphoinositides (Akthar *et al.*, 1983; Deshmukh *et al.*, 1984).

In subsequent experiments PdIns4*P* kinase was purified from cytosolic and particulate material from rat brain by means of ammonium sulphate precipitation, DEAE-cellulose column chromatography and preparative isoelectric focusing. Both in cytosol and in membranes the lipid kinase activity resided in a protein with an M_r of 45 000 and a pI of 5.8 (van Dongen *et al.*, 1984). Immunoprecipitation of this protein in various and partially purified PtdIns4*P* preparations by affinity-purified antibodies reduced enzyme activity, confirming the identity of the M_r 45 000 protein as PtdIns4*P* kinase (van Dongen, 1985). To investigate the modulation of this lipid kinase activity by the degree of phosphorylation of B-50 in a more direct manner, partially purified PtdIns4*P* kinase extracts from rat brain were studied in the presence of added purified B-50 preparations which differ in their degree of phosphorylation. In this reconstituted system, conditions could be found where phospho-B-50 (pB-50) reduced PtdIns4*P* kinase activity and B-50 did not (Zwiers *et al.*, 1985; van Dongen *et al.*, 1985). Since the B-50 protein has no detectable protein or lipid kinase/phosphatase activity itself, it has been suggested that the protein B-50 may be a modulator of PtdIns4*P* kinase in the presynaptic plasma membrane of rat brain (Gispen *et al.*, 1985*b*).

B-50 kinase and protein kinase C

During studies on the regulation of the phosphorylation of B-50, it was found that the relevant kinase was insensitive to cyclic AMP and cyclic GMP, but was inhibited by the presence of ACTH and stimulated by Ca^{2+}, whereas most likely calmodulin does not act as transducer of the Ca^{2+} ions to activate the kinase (Gispen *et al.*, 1979; Rodnight, 1982; Sörensen & Mahler, 1983). Extensive studies were carried out to compare the characteristics of B-50 kinase with that of the well-known cyclic, nucleotide-insensitive, Ca^{2+}- and phospholipid-sensitive protein kinase C, present both in brain cytosol and membranes (Inoue *et al.*, 1977; Kuo *et al.*, 1980; Kikkawa *et al.*, 1982).

In view of the similarities in apparent M_r, isoelectric point, substrate specificity, metal requirements, sensitivity to modulators, phospholipids and protease treatment, Aloyo *et al.* (1982, 1983) concluded that B-50 protein kinase is very similar if not identical to protein kinase C. These findings link B-50 via its B-50 kinase/protein kinase C to the rapidly growing interest in the role that diacylglycerol (DG) plays in membrane function.

B-50 as a feedback modulator in PtdIns4,5P_2 hydrolysis: functional implications

From a variety of evidence it has been suggested that the specific hydrolysis of PtdIns4,5P_2 into inositol-trisphosphate (InsP_3) and DG is a key event in transmembrane signal transduction and intracellular Ca^{2+} mobilization. The InsP_3 is thought to mobilize Ca^{2+} from internal stores other than mitochondria (Berridge & Irvine, 1984), where DG is known to activate protein kinase C (Nishizuka, 1984). Although in cells and tissues other than brain some other substrate proteins have been identified, presently the function of protein kinase C substrates is largely unknown. In Fig. 1, a model is presented that could account for the related events in synaptic protein and lipid metabolism, allowing B-50 to exert control by feedback inhibition of PtdIns4,5P_2 turnover. The validity of this model is presently a subject of further study in our laboratory. Several implications of this model are evident. First of all, the model predicts that in receptor-activated PtdIns4,5P_2 hydrolysis, the DG-stimulated protein kinase C will increase the degree of phosphorylation of B-50 thereby decreasing the activity of PtdIns4*P* kinase necessary to restore PtdIns4,5P_2 available for hydrolysis. Recent observations seem to question the primacy of Ca^{2+} in secretory control (see Baker, 1984). In fact, many secretory systems can be

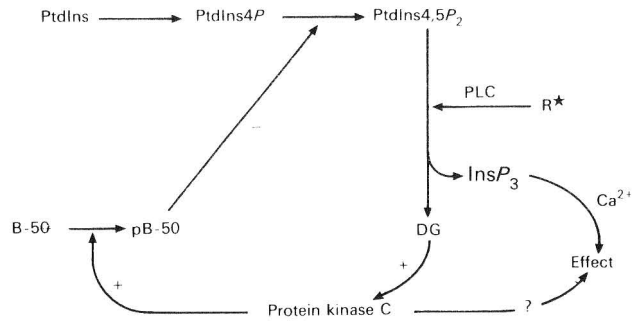

Fig. 1. *Working model for the role of phosphoprotein B-50 in the feedback control of phosphatidylinositol 4,5-bisphosphate turnover*

For an explanation see the text. Abbreviations: PtdIns, phosphatylinositol; R*, receptor; PLC, phospholipase C.

activated by agents that promote the breakdown of (poly)phosphoinositides, often with little change in intracellular free Ca^{2+}. Furthermore, it has been shown that DG can induce structural transitions in phospholipid bilayers that are crucial to the necessary membrane function in vesicular exocytosis (Das & Rand, 1984). Hence, the B-50 protein may be involved in the control of exocytosis from those presynaptic terminals in which it is localized.

In this respect it should be noted that neuropeptides have been shown to act presynaptically as modulators of neurotransmitter release (Versteeg, 1980; Mulder *et al.*, 1984; D. H. G. Versteeg & A. H. Mulder, unpublished work). Previously, it was shown that ACTH and congeners inhibit the phosphorylation of B-50 in rat brain synaptic plasma membranes (Zwiers *et al.*, 1978; Gispen & Zwiers, 1985). The model presented concerning a role for B-50 as a feedback modulator in PtdIns4,5P_2 hydrolysis, suggests that presynaptic modulation of neurotransmitter release by peptides may be brought about through changes in PtdIns4,5P_2 metabolism (Gispen *et al.*, 1985*a*,*b*).

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Role of the Golgi complex and characteristics of post-Golgi transport in the biosynthesis of intestinal microvillar proteins

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The small-intestinal enterocyte is a highly polarized cell which possesses a number of enzymes (mostly peptidases and glycosidases) functionally located in the apical (microvillar) portion of the plasma membrane (Kenny & Maroux, 1982). Due to the abundance of the microvillar enzymes, this cell type has become an attractive model system for studying eukaryotic plasma membrane protein biogenesis. Work performed in the last few years in several laboratories has revealed a common theme in the biosynthetic events of different microvillar enzymes. This includes a co-translational membrane insertion, high mannose glycosylation of the primary translation product in the rough endoplasmic reticulum and trimming and complex glycosylation of *N*-linked oligosaccharides (and probably *O*-linked glycosylation) in the Golgi complex before expression in the microvillar membrane (Danielsen *et al.*, 1984).

It has been unclear for some time, however, whether newly synthesized microvillar enzymes, as suggested by Quaroni *et al.* (1979) and Hauri *et al.* (1979), pass from the Golgi complex to the basolateral plasma membrane before insertion in the microvillar membrane. A related and more general problem, still unsolved, is at what stage the sorting of newly synthesized microvillar enzymes

Abbreviation used: SDS, sodium dodecyl sulphate.