Experimental Physiology (1997), **82**, 609–628
Printed in Great Britain

REVIEW ARTICLE

INTRACELLULAR TRAFFICKING OF PROHORMONES AND PRONEUROPEPTIDES: CELL TYPE-SPECIFIC SORTING AND TARGETING

MARCELO J. PERONE, SIMON WINDEATT AND MARIA G. CASTRO*

Molecular Medicine Unit, Department of Medicine, University of Manchester, Stopford Building, Room 1.302, Manchester M13 9PT, UK

(MANUSCRIPT RECEIVED 6 SEPTEMBER 1996, ACCEPTED 3 MARCH 1997)

CONTENTS PAGE

INTRODUCTION

Hormones and neuropeptides are usually synthesized as large precursor molecules which must undergo a series of post-translational modifications before they are released from secretory vesicles after stimulation by specific secretagogues. The classical vectorial transport of precursors and mature peptide products from the endoplasmic reticulum (ER) to their site of release has been the subject of intensive studies during the past 25 years, but there are a number of unresolved issues which still challenge cell biologists. The main issues are: (i) the molecular mechanisms underlying targeting and sorting of prohormones within the eukaryotic secretory pathway; (ii) further identification and intracellular site of action of post-translational processing enzymes; (iii) tissue-specific processing of hormone and neuropeptide precursors; and (iv) molecular mechanisms underlying intracellular transport of hormone and neuropeptide precursors and their cleavage products to alternative intracellular compartments besides the secretory pathway.

* To whom correspondence should be addressed.

Table 1. *Examples of neuropeptides and hormones originated from precursor protein*

Precursor	Peptide products	Reference
POMC	ACTH; α-MSH; endorphins; β-LPH	Nakanishi *et al.* (1979)
Proinsulin	Insulin	Smeekens *et al.* (1992)
ProVIP	VIP; PHM-27	Itoh *et al.* (1983)
Proenkephalin	Leu-enkephalin; Met-enkephalins	Comb *et al.* (1982)
ProNGF	Nerve growth factor	Seidah *et al.* (1996)
Proglucagon	Glucagon	Rothenberg *et al.* (1995)
ProNPY	Neuropeptide Y	Wulff *et al.* (1993)
ProCRH	CRH	Morrison *et al.* (1995)
ProCGRP	Calcitonin and calcitonin gene-related peptides	Johansen *et al.* (1991)
ProCCK	Cholecystokinin	Yoon *et al.* (1995)
Progastrin	Gastrin	Dickinson *et al.* (1995)
ProGnRH	GnRH	Ackland *et al.* (1988)
ProELH	ELH and ELH-derived peptide	Jung *et al.* (1993)
Provasopressin	AVP; neurophysin	Hyodo *et al.* (1992)
ProPTH	Parathyroid hormone	Hendy *et al.* (1995)
Proprolactin	Prolactin	Clapp *et al.* (1994)

Abbreviations: ACTH, adrenocorticotrophic hormone; AVP, arginine vasopressin; CCK, cholecystokinin; CRH, corticotrophin-releasing hormone; ELH, egg-laying hormone; GnRH, gonadotrophin-releasing hormone; β-LPH, β-lipotrophic hormone; α-MSH, α-melanocorticotrophic hormone; NGF, nerve growth factor; NPY, neuropeptide Y; PHM-27, amino-terminal histidine, carboxy-terminal methionine amide; POMC, pro-opiomelanocortin; PTH, parathyroid hormone; VIP, vasointestinal polypeptide.

We will review the endoproteolytic enzymes involved in cleaving prohormones and proneuropeptides within the secretory pathway. We will also discuss possible mechanisms involved in the intracellular trafficking and sorting of precursors within the secretory pathway, as well as their translocation to other intracellular compartments, particularly the nucleus and the cytoplasm. We will present some recent results from our laboratory and others which indicate the possibility of cell type-specific factors that might influence the intracellular fate of these peptide messengers.

POST-TRANSLATIONAL PROCESSING OF PRONEUROPEPTIDES AND PROHORMONES

Most prohormones and proneuropeptides contain within their amino acid sequence several peptide products with different biological activities (Table 1). For example, pro-opiomelanocortin (POMC) is the precursor molecule for adrenocortictrophin (ACTH), β-lipotrophin, β-endorphin and α-melanocyte-stimulating hormone (Nakanishi, Inoue, Kita, Nakamura, Chang, Cohen & Numa, 1979). Proenkephalin contains multiple copies of the same peptide, i.e. six copies of Met-enkephalin and one copy of Leu-enkephalin (Comb, Seeburg, Adelman, Eiden & Herbert, 1982). The precursor of Phe-Met-Arg-Phe (FMRF)-amide synthesized by the neurons of the mollusc *Aplysia californica* contains this tetrapeptide repeated 28 times within its amino acid sequence and a single copy of the related peptide Phe-Leu-Arg-Phe (FLRF)-amide (Taussig & Scheller, 1986). Vasoactive intestinal polypeptide (VIP) and amino-terminal histidine, carboxy-terminal methionine amide (PHM)-27 are also flanked by pairs of amino acids residues within their protein precursor, which also contains a putative

twenty-two amino acid signal peptide (Itoh, Obata, Yanaihara & Okamoto, 1983). Other prohormones and proneuropeptides contain only one biologically active peptide product within their sequence, e.g. proprolactin, proinsulin, among others (Table 1).

Endoproteolytic cleavage of prohormones and proneuropeptides is a key step in the generation of biologically active hormones and neuropeptides within cells of the endocrine and nervous systems, respectively. However, several other post-translational modifications, which affect the lateral chain of amino acid residues (glycosylation, amidation, acetylation etc.), are sometimes essential for their full biological activity, peptide stabilization and resistance to degradation in biological fluids.

Although uncleaved neuropeptide and hormone precursors were thought not to be biologically active, we have recently demonstrated that the full-length precursor for corticotrophin-releasing hormone (CRH) elicits the release of ACTH from primary cultures of rat anterior pituitary cells (Morrison, Tomasec, Linton, Lowry, Lowenstein & Castro, 1995) and induces cell proliferation of AtT20 (mouse corticotrophic) and CHO-K1 (Chinese hamster ovary) cells (Castro, Tomasec, Morrison, Murray, Hodge, Blanning, Linton, Lowry & Lowenstein, 1995*b*). There is now evidence for a novel biological activity of the gastrin precursor: a growth-promoting effect independent of that of the processed products has been demonstrated *in vivo* and *in vitro* (Wang, Koh, Varro, Cahill, Dangler, Fox & Dockray, 1996). This study suggests that the gastrin precursor may play a role in the proliferation of the colonic mucosa.

The biological role of the amino-terminal sequences for many precursor molecules remains to be elucidated. However, it has been demonstrated that the amino-terminal sequences of POMC and procalcitonin can exert mitogenic activity on the adrenal glands (Estivariz, Carino, Lowry & Jackson, 1988) and bone cells (Burns, Forstrom, Friday, Howard & Roos, 1989), respectively.

Several endoproteases have been postulated as prohormone-processing enzymes (Table 2). It has recently been demonstrated that many prohormones and proneuropeptides can be cleaved by the same processing enzymes. It is also possible to predict the putative sites of proteolytic processing within novel prohormones, e.g. paired basic amino acid residues such as lysine(K)–arginine(R), RR, RK or KK, although endoproteolysis at mono-, tri- and tetra-basic amino acid residues can also occur (Smeekens, 1993). Many enzymes belonging to the family of subtilisin/Kex2-serine proteases have the ability to process neuropeptide and hormone precursors endoproteolytically; however, prohormone convertase (PC)1 and PC2 are the best characterized candidates because they are selectively expressed in neuronal and endocrine cells and tissues (Seidah, Chretien & Day, 1994; Perone, Ahmed, Linton & Castro, 1996).

Two lines of evidence suggest that a few PCs could cleave a broad spectrum of prohormone and proneuropeptide precursors: (1) there is a wide distribution of PC1 and PC2 in neuronal and endocrine tissues expressing many different precursor molecules; and (2) these PCs seem to be highly preserved during evolution, since the endoproteolytic products of the egg-laying hormone (ELH), which is a prohormone synthesized within the bag cell neurons of the marine mollusc *Aplysia californica*, are similar when this prohormone is expressed in AtT20 cells (Jung, Kreiner & Scheller, 1993). Endoproteases may be affected and regulated by endogenous factors *in vivo*, which may be cell and species specific; these factors can either increase or inhibit processing of protein precursors. In that respect, it has recently been demonstrated that the intact form of the neuroendocrine-specific polypeptide 7B2 is a potent inhibitor of PC2 but not PC1 (Martens, Braks, Eib, Zhou & Lindberg, 1994), while processed 7B2 stimulates

Table 2. *Examples of eukaryotic proteases which cleave precursors of secretory peptides endoproteolytically*

Protease	Precursor	Reference
PC1	Pro-opiomelanocortin	Benjannet *et al.* (1991)
	Proneuropeptide Y; propancreatic polypeptide	Wulff *et al.* (1993)
	Proenkephalin	Breslin *et al.* (1993)
	Proglucagon	Rothenberg *et al.* (1995)
	Prodynorphin	Dupuy *et al.* (1994)
	Procholecystokinin	Yoon & Beinfeld (1995)
	Rat proinsulin I	Smeekens *et al.* (1992)
	Prorenin	Neves *et al.* (1996)
PC2	Pro-opiomelanocortin	Benjannet *et al.* (1991)
	Pro-islet amyloid polypeptide	Badman *et al.* (1996)
	Proenkephalin	Breslin *et al.* (1993)
	Proglucagon	Rothenberg *et al.* (1995); Rouille *et al.* (1994)
	Rat proinsulin I	Smeekens *et al.* (1992)
PACE 4	Pro-nerve growth factor	Seidah *et al.* (1996);
	Pro-von Willebrand factor	Creemers *et al.* (1993)
PC5	Pro-nerve growth factor	Seidah *et al.* (1996)
Furin	Proenkephalin	Breslin *et al.* (1993)
	Transforming growth factor β1	Dubois *et al.* (1995)
	Proalbumin	Brennan & Nakayama (1994)
	Pro-nerve growth factor	Seidah *et al.* (1996)
	Pro-von Willebrand factor	Van de Ven *et al.* (1990)
	Rat proinsulin I	Smeekens *et al.* (1992)
	Proparathyroid hormone	Hendy *et al.* (1995)
Kex2	Yeast pro α-mating factor	Julius *et al.* (1984); Egel-Mitani *et al.* (1990)
	Prosomatostatin I and II	Bourbonnais *et al.* (1994)
	Yeast exoglucanase	Basco *et al.* (1990)
PTP	Proenkephalin; proneuropeptide Y	Schiller *et al.* (1996)
YAP3p	pACTH(1–39); proinsulin; cholecystokinin; dynorphin A and B; amidorphin	Cawley *et al.* (1996)
	Prosomatostatin I and II	Cawley *et al.* (1993); Bourbonnais *et al.* (1994)
	Pro-opiomelanocortin	Azaryan *et al.* (1993)
Aspartic protease 70 kDa	Proenkephalin	Azaryan *et al.* (1995)
Cathepsin B	Prorenin	Neves *et al.* (1996)

Abbreviations: PACE 4, paired basic amino acid cleaving enzyme; PC, prohormone convertase; PTP, prohormone thiol protease; YAP3p, yeast aspartic protease 3.

POMC cleavage *in vitro*, probably activating PC2 (Braks & Martens, 1995). Also, changes in intracellular conditions, such as pH and Ca^{2+} concentration, might modulate endoproteolysis. Defined pH and Ca^{2+} concentrations are required within the trans Golgi network (TGN) for the autocatalytic activation of proPC1 and proPC2 (Shennan, Taylor, Jermany, Matthews & Docherty, 1995) to biologically active forms.

A vast number of chemical modifications of peptides and hormones has been described, but in only a few cases has their physiological significance been well documented (Guttman & Boissonnas, 1961; Bradbury, Finnie & Smyth, 1982; Akil, Shiomi & Matthews, 1985; Birch,

Estivariz, Bennett & Loh, 1991). Most post-translational modifications have been suggested to play essential intracellular functions, such as the provision of adequate sorting signals for targeting of hormones and neuropeptides to appropriate intracellular compartments (Hurtley, Bole, Hoover-Litty, Helenius & Copeland, 1989; Cool, Fenger, Snell & Loh, 1995). It has been demonstrated that N-linked glycosylated residues may reduce endoproteolysis of N-POMC(1–77) (Birch *et al.* 1991) and prorenin (Ladenheim, Seidah & Rougeon, 1991), although another report demonstrated that glycosylation does not play a significant role in POMC endoproteolysis (Noel, Keutmann & Mains, 1991). Post-translational modifications could play crucial extracellular roles by modifying the interaction of the neuropeptides and hormones with their receptors or by altering their half-lives in body fluids.

CELL TYPE-SPECIFIC TRANSLOCATION OF NEUROPEPTIDES AND HORMONES WITHIN EUKARYOTIC CELLS

Targeting and secretion of neuropeptides and hormones

Hormones and neuropeptides need to be exported from the cells in which they are synthesized in order to reach their target tissues and exert their biological actions; for this reason targeting, sorting and secretion of hormones and neuropeptides is a carefully controlled mechanism within endocrine and neuronal cells.

Secretory proteins, lysosomal proteins and constitutive membrane proteins share the first steps in their vectorial transport across the intracellular membranous compartments within eukaryotic cells. The signal-recognition particle (SRP) and its receptor (SR) are involved in the co-translational targeting of nascent protein–ribosome complexes to the ER in eukaryotyic cells. The SRP protein subunit that recognizes the signal peptide of nascent polypeptides and the SR subunits contain GTPase domains. They work as adaptors between the cytosol and the protein translocation apparatus in the ER membrane (Bacher, Lütcke, Jungnickel, Rapoport & Dobberstein, 1996). The signal peptide is cleaved by an ER endopeptidase before the pro-hormone or proneuropeptide is completely synthesized. Some secretory proteins lack a signal peptide, e.g. interleukin (IL)-1α and IL-1β (March, Moslely, Larsen, Cerreti, Braedt, Price, Gillis, Henney, Kronheim, Grabstein, Conlon, Hopp & Cosman, 1985), fibroblast growth factor (FGF)-1 (Jaye, Howk, Burgess, Ricca, Chier, Ravera, O'Brien, Modi, Maciag & Drohan, 1986), FGF-2 (Abraham, Mergia, Whang, Tumolo, Friedman, Hjerrild, Gospodarowicz & Fiddes, 1986), platelet-derived endothelial cell growth factor (Ishikawa, Miyazono, Hellman, Drexler, Werstedt, Hagiwara, Usuki, Takaku, Risau & Heldin, 1989), ciliary neurotrophic factor (Lin, Mismer, Lile, Armes, Butler, Vannice & Collins, 1990) and blood coagulation factor XIIIa. These proteins are released extracellulary, suggesting that an alternative pathway for protein secretion might exist within eukaryotic cells (Rubartelli, Cozzolino, Talio & Sitia, 1990). For exerting their biological activities, these proteins require post-translational modifications that are only performed by living cells, so the possibility that they could be released by cell lysis must be excluded. Transfected COS-1 (SV40 transformed African green monkey kidney) cells expressing FGF-2 have shown an ER–Golgi-independent and ATP-dependent export of FGF-2 (Florkiewicz, Majack, Buechler & Florkiewicz, 1995). Thus, secretion of FGF-2 appears to be different from that of other secretory factors lacking signal peptide. Energy-blocking agents seem to increase secretion of IL-1β and thioredoxin (Rubartelli, Bajetto, Allavena, Wollman & Satia, 1992). Although the molecular mechanisms involved in protein export remain unclear, the results cited here indicate that besides the classical division

of constitutive and regulated protein secretion, other types of secretory mechanisms must exist within eukaryotic cells. These would include the pathway used by peptides lacking classical signal sequences. In a recent study, Cleves, Cooper, Barondes & Kelly (1996) have shown an alternative pathway for protein secretion in yeast. This novel secretory mechanism is independent of a signal peptide and does not involve the yeast multidrug resistance-like transporter, Ste6p. The isolation of the genes involved in this alternative secretory mechanism may provide the tools for the identification of their mammalian counterparts.

Hormones and neuropeptides are released from endocrine and neuronal cells, respectively, via constitutive and/or regulated secretion (Gumbiner & Kelly, 11982). Proteins that exit the cell using the constitutive route are secreted immediately after they have been synthesized and exocytosis takes place at a constant rate without any external stimulus. Constitutive secretion may be blocked by inhibiting protein synthesis (Schmidt & Moore, 1994). On the other hand, within the regulated secretory pathway, secreted proteins can be stored at high concentrations for long periods of time within secretory vesicles, before they are released. Even in the absence of protein synthesis the release of a given hormone or neuropeptide is triggered by an external stimulus, which can in turn change second messenger levels.

The presence of distinct secretory pathways within the same cell requires a sorting mechanism to transport secretory proteins to the correct route from the ER to Golgi apparatus and finally to secretory granules destined for regulated secretion. The trans-most cisternae of the Golgi complex, a clathrin-coated compartment, is the site where sorting of proteins destined for either the regulated or the constitutive secretory pathway takes place (Orci, Ravazzola, Amherdt, Perrelet, Powell, Quinn & Moore, 1987; Tooze & Huttner, 1990). Neuronal and endocrine cells, being highly specialized secretory cells, must separate proneuropeptides and prohormones from other proteins, such as membrane proteins or lysosomal hydrolases. To date, the mechanism involved in sorting of proteins destined for the regulated secretory pathway has not been elucidated. Sorting domains of most regulated secretory proteins reside in their N-terminal region, immediately after the signal sequence responsible for ER translocation (Cool *et al.* 1995). Comparison of amino acid sequences within this region from different secretory proteins does not show a consensus sequence; however, their hydrophobic characteristics and secondary structures appear to be the key elements for sorting, and these are absent in constitutively secreted proteins (Gorr & Darling, 1995). It has recently been shown that the domain responsible for the targeting of POMC to the regulated secretory pathway resides within its N-terminal region, which contains an amphipathic loop of thirteen amino acids. Sorting to the regulated secretory pathway is dependent on the integrity of the disulphide bridge between amino acids Cys_8–Cys_{20} (Cool *et al.* 1995). It has also been demonstrated that the N-terminal region of prosomatostatin contains information for targeting to the regulated secretory pathway within transfected mammalian endocrine cell lines (Sevarino, Stork, Ventimiglia, Mandel & Goodman, 1989). Another possibility to explain the sorting of proneuropeptides and prohormones is that sorting domains could be recognized by a membrane-anchored receptor/carrier at the trans-most cisternae of the Golgi complex. A group of 25 kDa proteins that bind selectively to prolactin, insulin and human growth hormone but do not bind to proteins which are not sorted to the regulated secretory pathway were identified and called 'sortases' (Chung, Walter, Aponte & Moore, 1989). However, Gorr and co-workers characterized this set of proteins as chymotrypsinogen A or B (Gorr, Hamilton & Chon, 1992). Recently, Cool and co-workers have identified a sorting receptor, i.e. membrane-associated carboxypeptidase E (CPE), in pituitary Golgi-enriched and secretory

granule membranes (Cool, Normant, Shen, Chen, Pannell, Zhang & Loh, 1997). Protein aggregation might also be a mechanism capable of sorting proteins to the regulated secretory pathway from resident and constitutively secreted proteins (Tooze, Kern, Fuller & Howell, 1989).

Endoproteolytic processing of prohormones might also affect their subsequent sorting and the intracellular fate of the cleavage peptides; this was demonstrated in the case of ELH prohormone expressed in AtT20 cells (Jung *et al.* 1993). After endoproteolytic cleavage, amino- and carboxy-terminal-derived peptides from the ELH prohormone were targeted and packaged into different subpopulations of secretory vesicles (Sossin, Sweet-Cordero & Scheller, 1990). A recent study attributes an essential role of one cleavage site to the correct sorting of prorenin, expressed in AtT20 cells, to the regulated or constitutive secretory pathway (Brechler, Chu, Baxter, Thibault & Reudelhuber, 1996). A point mutation at the cleavage site between the prosegment and renin changes the fate of prorenin from the regulated to the constitutive pathway.

In somatomammotrophic cells, prolactin and growth hormone are packaged into separate vesicles (Fumagalli & Zanini, 1985). This indicates that both hormones possess distinguishable signals whereby the sorting machinery of a particular cell type is able to differentially segregate these peptides to different vesicle populations. Our results using AtT20 cells, after neuronal differentiation, suggest that prohormones and proneuropeptides expressed in eukaryotic neurons could also be packaged into different subsets of secretory vesicles and secreted differentially in response to an extracellular stimulus (Castro, Brooke, Bullman, Hannah, Glynn & Lowry, 1991; M. J. Perone & M. G. Castro, unpublished observations). We used transfected AtT20 cells expressing human proCRH and endogenous POMC; these cells must be able to direct ACTH and proCRH-derived peptides into different secretory granule populations since the mature peptides could be differentially released in response to an extracellular stimulus. It has been demonstrated that different intracellular mechanisms involving second messengers could mediate the release of different sets of secretory granules containing peptides. Many other neuropeptides have been found to co-localize within secretory granules of neurones, e.g. calcitonin gene-related peptide (CGRP) and tachykinins, CGRP and somatostatin, somatostatin and tachykinins (Merighi, Polak, Gibson, Gulbenkian, Valentino & Peirone, 1988), and substance P and CGRP (Skofitsch & Jacobowitz, 1985). Neuropeptide co-localization within secretory granules of neurones suggests that they could also be co-released in response to an external stimulus. The simultaneous release of tachykinins and CGRP has been observed *in vitro* (Saria, Gamse, Petermann, Fischer, Theodorsson-Norheim & Lundberg, 1986).

The vectorial transport of neuropeptides and hormones within neuronal and endocrine cells, respectively, might be governed by the polarized distribution of the Golgi apparatus within these cells (Lowenstein, Morrison, Bain, Shering, Banting, Douglas & Castro, 1994). Secretory vesicles containing hormones might travel towards the cell surface associated with and, perhaps, guided by microtubules and/or microfilaments. This assumption is sustained by experiments employing drugs which disrupt the microtubule–microfilament network of cells in culture. The role of the cytoskeleton in the secretion of prohormones and proneuropeptides has remained unclear. Novick & Botstein (1985) demonstrated that lack of actin can promote an accumulation of secretory vesicles, suggesting a role for actin during late stages of the secretory pathway in yeast. Our studies in AtT20 cells expressing proCRH suggest that disruption of neither microtubules nor microfilaments affects the constitutive release of CRH(1–41). We observed that actin filaments play a role in mediating the release

Table 3. *Intracellular localization of neuropeptides, hormones, growth factors and cytokines*

Secretory protein	Cell type	Subcellular distribution N	M	C	Reference
GH	Somatotroph, lactotroph, gonadotroph	+	+	+	Mertani *et al.* (1996)
	Hepatocyte	+	–	+	Lobie *et al.* (1994)
Glutathione-related enzymes	Granule cell	–	+	+	Huang & Philbert (1995)
	Astrocyte	–	+	+	Huang & Philbert (1995)
Apolipoprotein J/clusterin	Hepatoma cell line	+	–	+	Reddy *et al.* (1996)
Prolactin	Lactotroph	+	+	+	Giss & Walker (1985)
Procathepsin L	*ras*-transformed mouse fibroblast	+	–	+	Hiwasa & Sakiyama (1996)
Proenkephalin	Rodent and human embryonic fibroblast, rodent myoblast	+	–	+	Böttger & Spruce (1995)
Fibroblast growth factor 3	Epithelial cell line	+	–	+	Kiefer & Dickson (1995)
FHF-1 and 2	HEK (293) cell	+	–	+	Patry *et al.* (1994)
GnRH	Gonadotroph	+	–	+	Morel *et al.* (1994)
TRH	Lactotroph	+	–	+	Morel (1994)
Somatostatin	Lactotroph	+	–	+	Morel (1994)
BRCA1	Mammary epithelial cell	+	–	+	Jensen *et al.* (1996)
FGF-1	NIH 3T3	+	–	+	Lin *et al.* (1996)
ProCRH	CHO-K1	+	–	+	Castro *et al.* (1995*b*)
Insulin	Hepatoma cell line	+	–	+	Smith & Jarett (1990)
Interleukin-1	Murine thymoma cell line	+	–	+	Solari *et al.* (1994)

Abbreviations: N, nucleus; M, mitochondria; C, cytosol; BRCA-1, familial breast and ovarian cancer gene; FGF, fibroblast growth factor; FHF, fibroblast growth factor homologous factor; GH, growth hormone; GnRH, gonadotrophin-releasing hormone; HEK, human embryonic kidney; TRH, thyrotropin-releasing hormone.

of CRH(1–41) through the regulated secretory pathway in transfected AtT20 cells (M. J. Perone & M. G. Castro, unpublished observations). To date, nothing is known about the numerous associated microtubule–microfilament proteins which are, perhaps, the true motors in vesicular trafficking within eukaryotic secretory cells (i.e. endocrine cells and neurons).

From all the work reviewed above, perhaps one of the most striking conclusions is that the intracellular machinery for prohormone sorting and secretion is probably highly conserved across a wide spectrum of different cell types, even in cells as evolutionarily distant as yeast and neurons (Bennett & Scheller, 1993). This is exemplified by ELH prohormone, which is endoproteolytically processed and sorted in a similar manner in AtT20 cells and in the bag cell neurons of the mollusc *A. californica* (Jung *et al.* 1993).

Translocation of neuropeptides and hormones to more than one subcellular compartment

It has been shown that different neuropeptide and hormone products encoded by a single gene can be located to more than one intracellular compartment (Table 3; Burwen & Jones, 1987; Morel, 1994; Castro & Morrison, 1997). Some of the molecular mechanisms by which this can be achieved are: (a) alternative transcription initiation; (b) alternative translation initiation; and (c) alternative RNA splicing.

Alternative transcription initiation. When alternative initiation sites for transcription are used, the presence of alternative promoters will give rise to transcripts that differ only in the composition of their 5′ ends. If the gene contains in-frame translation initiation sites (usually ATG codons), it can give rise to proteins which will differ at the N-terminal. Since this is the region in which ER and mitochondrial localization signals are usually present, the resulting proteins could be targeted to different intracellular compartments. The use of these alternative transcription sites could therefore produce an extended or truncated version of a given protein. If a gene encoding for a protein that is usually secreted has a potential in-frame downstream transcription initiation site, a truncated version of it may be produced which will not possess a signal peptide and so it will not be translocated into the rough ER. This protein may then be targeted to another intracellular compartment, such as the nucleus or the cytosol. Examples of proteins that are synthesized using alternative initiation of transcription include the *Saccharomyces cerevisiae* enzyme invertase (Carlson, Taussig, Kustu & Botstein, 1983) and carnitine acetyltransferase (Corti, Didonato & Finocchiaro, 1994), and gelsolin, which is produced by vertebrate cells (Kwiatkowski, Mehl & Yin, 1988). To date this mechanism has not been described for neuropeptides or hormones, but it is possible that it may be used by eukaryotic cells expressing neuropeptide or hormone genes in which the peptide products are targeted to other subcellular compartments besides the classical secretory pathway.

Alternative translation initiation. Alternative initiation of translation can occur when there are two potential in-frame initiation sites for translation. There must be two or more in-frame initiation codons within a given mRNA transcript. If translation occurs from the classical initiation site the polypeptide chain produced will contain an intact signal peptide which targets the protein to the secretory pathway. However, if translation initiation occurs from an in-frame downstream or upstream initiation site, then a truncated or elongated version of the protein is synthesized and the signal peptide is either truncated or modified by an extended N-terminal stretch of amino acids which can mask or override the signal peptide. The protein is therefore not able to enter the ER. Use of this mechanism can explain how proteins that would normally go through the secretory pathway could be translocated to other subcellular compartments within the cell, such as the nucleus, mitochondria or cytoplasm. This mechanism has been described for growth factors, e.g. in transfected COS-1 cells and reticulocyte lysates, FGF-3 is translated almost entirely from an upstream CUG codon instead of the classical AUG codon and this results in the FGF-3 being located to two different subcellular compartments, the nucleus and the secretory pathway. In this case, the long polypeptide form is targeted to the nucleus and the shorter form that has an N-terminal signal peptide is targeted to the secretory pathway. Owing to the competition between the nuclear localization and secretory signals, the proportion of FGF-3 present in the different compartments depends on the strength of the corresponding translation initiation sites (Kiefer, Acland, Pappin, Peters & Dickson, 1994).

FGF-2 has also been found to have alternative codons for translation initiation (Amalric, Baldin, Bosc-Bierne, Bugler, Couderc, Guyader, Patry, Prats, Roman & Bouche, 1991). A truncated form is produced by initiation of translation at a downstream in-frame initiation codon. The resulting protein does not contain a signal peptide so it cannot enter the ER. FGF-2 has been shown to be targeted to the nucleus in a variety of cell types, but this appears to involve a specific receptor-mediated interaction that occurs in the cytoplasm. Heparan sulphate proteoglycans are involved in the internalization and nuclear translocation of FGF-2 (Amalric, Bouche, Bonnet, Brethenou, Roman, Trucheet & Quarto, 1994). It has also been shown by Spence, Sheppard, Davie, Matuo, Nichi, McKeehan, Dodd & Matusik (1989) that

rat probasin, a protein produced by the prostate, is secreted and also localized to the nucleus. This is due to translational initiation at an alternative in-frame downstream AUG codon, giving rise to a truncated version of the probasin protein. This protein lacks a signal peptide which means that it cannot enter the ER.

An alternative translation initiation mechanism has recently been described for procorticotrophin-releasing hormone and proenkephalin. Work done in our laboratory has demonstrated that the intact CRH precursor, which contains a signal peptide, is translocated to the secretory pathway and also to the nucleus within stably transfected CHO-K1 cells (Castro *et al.* 1995*b*; Castro & Morrison, 1995; Morrison *et al.* 1995). Nuclear proCRH is in close association with double-stranded DNA, suggesting that it might play a role in the regulation of gene expression (Castro, Morrison, Tomasec, Linton & Lowenstein, 1995*a*). This abnormal nuclear localization of proCRH is probably due to the use of alternative initiation codons for translation. The truncated version of proCRH is initiated at an in-frame downstream initiation codon and could be translocated to the nucleus, since it has a truncated signal peptide which cannot target the precursor molecule to the ER. The intact pre-proCRH molecule, containing an intact signal peptide, is targeted to the secretory pathway within CHO-K1 cells (Morrison *et al.* 1995). Böttger & Spruce (1995) have demonstrated nuclear localization of the proenkephalin precursor in rodent and human embryonic fibroblast cell lines (Swiss 3T3 and MRC-5 cells) and in rodent myoblast cells (C2C12 cells). They showed that when wild-type proenkephalin was expressed in COS cells, it was localized exclusively in the cytoplasm, while proenkephalin that was mutated at the first ATG codon or devoid of its signal peptide was targeted to the nucleus as well as the cytoplasm. Results from our laboratory using CHO cells expressing proenkephalin (Lindberg, Shaw, Finley, Leone & Deininger, 1991) show only cytoplasmic localization for immunoreactive (IR)-enkephalin as assessed using immunofluorescence techniques and confocal microscopy (M. G. Castro & I. Lindberg, unpublished observations). These results indicate that translocation of neuropeptide precursors is cell type specific, since different cell types might use different molecular mechanisms to biosynthesize neuropeptides or hormones. This will give rise to different molecular forms of the peptide products derived from a single gene, which could in turn be translocated to different subcellular compartments (Danpure, 1995).

Alternative splicing. Alternative splicing occurs at the pre-mRNA level and, instead of producing peptides that are different at their N-terminal sequences, it can give rise to peptides that contain different targeting amino acid sequences located anywhere within the protein. An example of this mechanism is the Ca^{2+}–calmodulin-dependent protein kinases (CAM), which mediate the action of intracellular calcium. δ-CAM is encoded by a single gene, which gives rise to a single pre-mRNA transcript that can be alternatively spliced to produce mRNAs containing different internal 'variable' regions. The alternative splicing produces three isoforms, two of which are cytosolic (δ_A and δ_C), while the δ_B isoform is targeted to the nucleus of mammalian cells. The δ_B isoform is targeted to the nucleus owing to the presence of a nuclear localization signal, which is started in the conserved region and finished by residues provided by the δ_B variable region (Srinivasan, Edman & Schulman, 1994).

CGRP is produced by alternative processing of RNA transcripts form the calitonin gene with the other product being the hormone calcitonin. Different mRNA transcripts predominate in different tissues, e.g. the CGRP-specific mRNA predominates in the hypothalamus and the calcitonin mRNA predominates in the thyroid (Amara, Jonas, Rosenfeld, Ong & Evans, 1982). In the substance P prohormone, seven exons are joined in alternate configurations,

generating precursors which contain two tachykinins (substance P and substance K) or only substance P (Nawa, Kotani & Nakanishi, 1984). The mechanism of alternative RNA splicing helps to increase the diversity of neuroendocrine gene expression products originated from a single gene, which might be translocated to different subcellular compartments.

Use of specific targeting sequences

The information for the translocation of neuropeptides, hormones and other secretory proteins, such as growth factors and cytokines, to the secretory pathway resides in the N-terminal amino acid sequence, i.e. signal peptide. The recent localization of proteins that were classically thought to be located exclusively within organelles of the secretory pathway to another subcellular location, i.e. the nucleus, has raised the possibility that these proteins might contain other specific targeting domains within their primary amino acid sequence. This gives rise to the possibility of competition between different targeting sequences. Usually, targeting sequences located at the N-terminus of a given protein prevail over targeting sequences located at other locations, i.e. internal or at the C-terminus. In this review we will only discuss nuclear localization signals (NLSs), since the nucleus is the other most common location for neuropeptides and hormones besides the secretory pathway.

Nuclear localization signals. NLSs determine protein movement and translocation across the nuclear membrane. They were first identified over two decades ago by using mutation/deletion analysis and by their capacity to cause normally cytoplasmic proteins to be transported to the nucleus. A classical NLS is present in the SV40 large tumour antigen: PKKKRKV (Kalderon, Richardson, Markham & Smith, 1984). Any mutation in this sequence will prevent the protein being transferred to the nucleus. Sequence homology has been used to identify other NLSs that could be present in other proteins and one of these is the 'bipartite NLS' that comprises two clusters of basic amino acids separated by a spacer region (Robbins, Dilworth, Laskey & Dingwall, 1991). The bipartite NLS can be found in the human testis, determining SRY gene product (also termed p27SRY) (Poulat, Girard, Chevron, Goze, Rebillard, Calas, Lamb & Berta, 1995), interferon-γ (Bader & Weitzerbin, 1994) and FGF-3 (Kiefer *et al.* 1994).

Work done with growth factors might bring some insights into the molecular mechanisms that mediate nuclear translocation of neuropeptides and growth hormones, as well as to their physiological roles within the nucleus. It has been demonstrated that any point deletion in the nuclear localization sequence of FGF-1 will cause it to fail to stimulate *in vitro* DNA synthesis and cell proliferation (Imamura, Engleka, Zhan, Tokita, Forough, Roeder, Jackson, Maier, Hla & Maciag, 1990). However, it is still able to bind to the FGF receptor and stimulate c-*fos* expression and receptor-mediated tyrosine phosphorylation. This demonstrates the potential signalling importance of nuclear localization sequences in growth factors that stimulate mitogenesis. It has also been demonstrated that the nuclear translocation of FGF-1 and FGF-2 occurs at specific stages of the cell cycle, e.g. for FGF-2 the nuclear translocation corresponds to the late G1 phase of the cell cycle (Baldin, Roman, Bosc-Bierne, Amalric & Bouche, 1990; Shiurba, Jing, Sakakura & Godsave, 1991).

A number of neuropeptides, prohormones and growth factors have been found in the nucleus, including insulin (Oldfine, Jones, Hradek, Wong & Mooney, 1978), angiotensin II (Re, Vizard, Brown & Bryan, 1984), human chorionic gonadotrophin (Rao & Mitra, 1979; Rajendran & Menon, 1983), gonadotrophin-releasing hormone (Zolman & Theodoropoulos, 1984), growth hormone, luteinizing hormone-releasing hormone (Millar, Rosen, Badminton, Pasqualini & Kerdelhue, 1983), proenkephalin (Böttger & Spruce, 1995), procorticotrophin-

releasing hormone (Castro *et al.* 1995*a*,*b*), vasoactive intestinal polypeptide, nerve growth factor (Yanker & Shooter, 1979), epidermal growth factor (Savion, Vlodavsky & Gospodarowicz, 1981), platelet-derived growth factor (Rakwicz-Szulczynska, Rodeck, Herlyn & Koprowski, 1986) and the β- and γ-interferons (Macdonald, Kushnaryov, Sedmark & Grossberg, 1986). Peptide hormones, including thyrotropin-releasing hormone and somatostatin (Gourdji, Tixier-Vidal, Morin, Pradelles, Morgat, Fromageot & Kerdelhue, 1973; Morel, 1994), were found to localize in the nucleus in lactotrophic cells.

The molecular mechanisms by which hormones, growth factors and neuropeptides are translocated to the nucleus of the cells in which they are synthesized or of target cells are not fully understood. Recently, a sugar-dependent process for nuclear import of glycoproteins has been reported (Duverger, Pellerin-Mendes, Mayer, Roche & Monsigny, 1995); this observation opens an interesting avenue for exploration of the role of carbohydrate in the mechanism of nuclear internalization of prohormones and proneuropeptides. Possible routes for the occurrence of nuclear localization of proteins that do not contain an identifiable nuclear localization sequence include: (a) peptide lacking a signal sequence, which would prevent its entry to the ER, could bind to a specific cytoplasmic binding protein containing a nuclear localization sequence and this could result in nuclear translocation; and (b) endocytotic vesicles containing a given neuropeptide or hormone could fuse with the outer nuclear membrane, thereby escaping the lysosomal degradation pathway. In this case, transport of the protein across the inner nuclear membrane would still be required. Nuclear localization could still occur if a given prohormone, proneuropeptide or growth factor was released. After secretion it could be internalized via specific receptors localized at the plasma membrane and translocated to the nucleus in the same cell (autocrine action), an adjacent cell (paracrine action) or a target cell located in a distant tissue or organ (endocrine action) (Hopkins, 1994). Nuclear translocation of neuropeptides and hormones raises the important question of the physiological implications of this novel intracellular localization for these peptide messengers and also opens up novel pathways of intracellular communication within the vertebrate endocrine and nervous systems.

CONCLUSION

In this review we have discussed novel secretory and targeting mechanisms for neuropeptide and hormone precursor molecules. Some mechanisms of secretion have been conserved between bacteria and higher eukaryotic mammalian cells, e.g. secretion of peptides lacking classical signal sequences, which may be mediated by ATP-driven translocators (Kuchler & Thorner, 1990). Neuropeptide and hormone precursor molecules lacking a signal peptide could be generated by the use of alternative initiation codons for translation. In this case, the cytoplasm of endocrine and nerve cells can provide a reservoir for secretory neuropeptides and hormones, respectively. The classical mechanism of secretion for neuropeptides and hormones is via the constitutive and/or the regulated secretory pathways. These processes involve trafficking and sorting within membrane-deliminated compartments in eukaryotic cells.

The sorting mechanism that determines the translocation of neuropeptides and hormones to the regulated secretory pathway has been the subject of intensive research during the past 15 years. Recently, membrane-associated carboxypeptidase has been identified as a sorting receptor for POMC within pituitary cells (Cool *et al.* 1997). There is also a growing body of evidence which shows the presence of neuropeptides and hormones within the nucleus.

Nuclear translocation could be mediated by a nuclear localization signal and could take place within the cell in which the neuropeptide or hormone is being synthesized, or it could be mediated by endocytosis via specific receptors in target cells.

The results summarized in this review indicate that neuropeptides and hormones, which were classically thought to be destined for secretion to act on distant target cells via specific receptors, can also be translocated to the nucleus and the cytoplasm of the cells in which they are synthesized. This expands the possible physiological actions of these peptide neurotransmitters in the nucleus, where they could regulate gene expression, and also in the cytoplasm, from where they could be released and act on the same or neighbouring cells in an autocrine or paracrine fashion, respectively.

This work is supported by the Biotechnology and Biological Sciences Research Council, Chemicals and Pharmaceuticals Directorate through a project grant to M.G.C. We also acknowledge the support which our laboratory receives from The Wellcome Trust, The Cancer Research Campaign, The Parkinson's Disease Society, The Royal Society, REMEDI, The Sir Halley Stewart Trust, SANDOZ Foundation for Gerontological Research and The Faculty of Medicine Bequest Fund, University of Manchester. We thank Professors A. M. Heagerty and S. Tomilson for their support and encouragement.

REFERENCES

ABRAHAM, J. A., MERGIA, A., WHANG, J. L., TUMOLO, A., FRIEDMAN, J., HJERRILD, K. A., GOSPODAROWICZ, D. & FIDDES, J. C. (1986). Nucleotide sequence of a bovine clone encoding the angiogenic protein basic fibroblast growth factor. *Science* **233**, 545–548.

ACKLAND, J. F., NIKOLICS, K., SEEBURG, P. H. & JACKSON, I. M. (1988). Molecular forms of gonadotropin-releasing hormone associated peptide (GAP): changes within the rat hypothalamus and release from hypothalamic cells in vitro. *Neuroendocrinology* **48**, 376–386.

AKIL, H., SHIOMI, H. & MATTHEWS, J. (1985). Induction of the intermediate pituitary by stress: synthesis and release of nonopioid form of beta-endorphin. *Science* **227**, 424–426.

AMALRIC, F., BALDIN, V., BOSC-BIERNE, I., BUGLER, B., COUDERC, B., GUYADER, M., PATRY, V., PRATS, H., ROMAN, A. M. & BOUCHE, G. (1991). Nuclear translocation of basic fibroblast growth factor. *Annals of the New York Academy of Sciences* **638**, 127–138.

AMALRIC, F., BOUCHE, G., BONNET, H., BRETHENOU, P., ROMAN, A. M., TRUCHEET, I. & QUARTO, N. (1994). Fibroblast growth factor-2 (FGF-2) in the nucleus: translocation process and targets. *Biochemical Pharmacology* **47**, 111–115.

AMARA, S. G., JONAS, V., ROSENFELD, M. G., ONG, E. S. & EVANS, R. M. (1982). Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature* **298**, 240–244.

AZARYAN, A. V., FRIEDMAN, T. C., CAWLEY, N. X. & LOH, Y. P. (1995). Characteristics of YAP3, a new prohormone processing aspartic protease from *S. cerevisae*. *Advances in Experimental Medicine and Biology* **362**, 569–572.

AZARYAN, A. V., WONG, M., FRIEDMAN, T. C., CAWLEY, N. X., TIVARIZ, F. E., CHAN, H. C. & LOH, Y. P. (1993). Purification and characterization of a paired basic residue-specific yeast aspartic protease encoded by the YAP3 gene. Similarity to the mammalian pro-opiomelanocortin converting enzyme. *Journal of Biological Chemistry* **268**, 11968–11975.

BACHER, G., LÜTCKE, H., JUNGNICKEL, B., RAPOPORT, T. A. & DOBBERSTEIN, B. (1996). Regulation by the ribosome of the GTPase of the signal-recognition particle during protein targeting. *Nature* **381**, 248–251.

BADER, T. & WEITZERBIN, J. (1994). Nuclear accumulation of interferon gamma. *Proceedings of the National Academy of Sciences of the USA* **91**, 11831–11835.

BADMAN, M. K., SHENNAN, K. I. J., JERMANY, J. L., DOCHERTY, K. & CLARK, A. (1996). Processing of pro-islet amyloid polypeptide (proIAPP) by the prohormone convertase PC2. *FEBS Letters* **378**, 227–231.

BALDIN, V., ROMAN, A.-M., BOSC-BIERNE, I., AMALRIC, F. & BOUCHE, G. (1990). Translocation of bFGF to the nucleus is G1 phase cell cycle specific in bovine aortic endothelial cells. *EMBO Journal* **9**, 1511–1517.

BASCO, R. D., GIMENEZ-GALLEGO, G. & LARRIBA, G. (1990). Processing of yeast exoglucanase (beta-glucosidase) in a kex2-dependent manner. *FEBS Letters* **268**, 99–102.

BENJANNET, S., RONDEAU, N., DAY, R., CHRETIEN, M. & SEIDAH, N. G. (1991). PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. *Proceedings of the National Academy of Sciences of the USA* **88**, 3564–3568.

BENNETT, M. K. & SCHELLER, R. H. (1993). The molecular machinery for secretion is conserved from yeast to neurons. *Proceedings of the National Academy of Sciences of the USA* **90**, 2559–2563.

BIRCH, N. P., ESTIVARIZ, F. E., BENNETT, H. P. J. & LOH, Y. P. (1991). Differential glycosylation of N-POMC1–77 regulates the production of γ3-MSH by purified pro-opiomelanocortin converting enzyme. *FEBS Letters* **290**, 191–194.

BÖTTGER, A. & SPRUCE, B. A. (1995). Proenkephalin is a nuclear protein responsive to growth arrest and differentiation signals. *Journal of Cell Biology* **130**, 1251–1262.

BOURBONNAIS, Y., GERMAIN, D., ASH, J. & THOMAS, D. Y. (1994). Cleavage of prosomatostatins by the yeast yap3 and kex2 endoprotease. *Biochimie* **76**, 226–233.

BRADBURY, A. F., FINNIE, M. D. & SMYTH, D. G. (1982). Mechanism of C-terminal amide formation by pituitary enzymes. *Nature* **298**, 686–688.

BRAKS, J. A. M. & MARTENS, G. J. M. (1995). The neuroendocrine chaperone 7B2 can enhance in vitro POMC cleavage by prohormone convertase PC2. *FEBS Letters* **371**, 154–158.

BRECHLER, V., CHU, W. N., BAXTER, J. D., THIBAULT, G. & REUDELHUBER, T. L. (1996). A protease processing site is essential for prorenin sorting to the regulated secretory pathway. *Journal of Biological Chemistry* **271**, 20636–20640.

BRENNAN, S. O. & NAKAYAMA, K. (1994). Cleavage of proalbumin peptides by furin reveals unexpected restrictions at the P2 and P'1 sites. *FEBS Letters* **347**, 80–84.

BRESLIN, M. B., LINDBERG, I., BENJANNET, S., MATHIS, J. P., LAZURE, C. & SEIDAH, N. G. (1993). Differential processing of proenkephalin by prohormone convertases 1 (3) and 2 and furin. *Journal of Biological Chemistry* **268**, 27084–27093.

BURNS, D. M., FORSTROM, J. M., FRIDAY, K. E., HOWARD, G. A. & ROOS, B. A. (1989). Procalcitonin's amino-terminal cleavage peptide is a bone-cell mitogen. *Proceedings of the National Academy of Sciences of the USA* **86**, 9519–9523.

BURWEN, S. J. & JONES, A. L. (1987). The association of polypeptide hormones and growth factors with the nuclei of target cells. *Trends in Biochemical Sciences* **12**, 159–162.

CARLSON, M., TAUSSIG, R., KUSTU, S. & BOTSTEIN, D. (1983). The secreted form of invertase in *Saccharomyces cerevisiae* is synthesised from mRNA encoding a signal sequence. *Molecular and Cellular Biology* **3**, 439–447.

CASTRO, M. G., BROOKE, J., BULLMAN, A., HANNAH, M., GLYNN, B. P. & LOWRY, P. J. (1991). Biosynthesis of corticotrophin-releasing hormone (CRH) in mouse corticotrophic tumour cells expressing the human proCRH gene: intracellular storage and regulated secretion. *Journal of Molecular Endocrinology* **7**, 97–104.

CASTRO, M. G. & MORRISON, E. (1995). Nuclear translocation of neuropeptides: possible nuclear roles. *Journal of Endocrinology* **146**, 9–13.

CASTRO, M. G. & MORRISON, E. (1997). Posttranslational processing of proopiomelanocortin in the pituitary and in the brain. *Critical Reviews in Neurobiology* **11**, 35–57.

CASTRO, M. G., MORRISON, E., TOMASEC, P., LINTON, E. A. & LOWENSTEIN, P. R. (1995*a*). Co-localisation of autoimmune antibodies specific for double stranded DNA with procorticotrophin-releasing hormone within the nucleus of stably transfected CHO-K1 cells. *Cell and Tissue Research* **282**, 367–376.

CASTRO, M. G., TOMASEC, P., MORRISON, E., MURRAY, C. A., HODGE, P., BLANNING, P., LINTON, E., LOWRY, P. J. & LOWENSTEIN, P. R. (1995*b*). Mitogenic effects and nuclear localisation of procorticotrophin-releasing hormone expressed within stably transfected fibroblast cells (CHO-K1). *Molecular and Cellular Endocrinology* **107**, 17–27.

CAWLEY, N. X., CHEN, H.-C., BEINFELD, M. C. & LOH, Y. P. (1996). Specific and kinetic studies on the cleavage of various prohormone mono- and paired-basic residue sites by yeast aspartic protease 3. *Journal of Biological Chemistry* **271**, 4168–4176.

CAWLEY, N. X., NOE, B. D. & LOH, Y. P. (1993). Purified yeast aspartic protease 3 cleaves anglerfish pro-somatostatin I and II at di- and monobasic sites to generate somatostatin-14 and 28. *FEBS Letters* **332**, 273–276.

CHUNG, K.-N., WALTER, P., APONTE, G. W. & MOORE, H.-P. H. (1989). Molecular sorting in the secretory pathway. *Science* **243**, 192–197.

CLAPP, C., TORNER, L., GUTIERREZ-OSPINA, G., ALCANTARA, E., LOPEZ-GOMEZ, F. J., NAGANO, M., KELLY, P. A., MEJIA, S., MORALES, M. A. & MARTINEZ DE LA ESCALERA, G. (1994). The prolactin gene is expressed in the hypothalamic–neurohypophyseal system and the protein is processed into a 14-kDa fragment with activity like 16-kDa prolactin. *Proceedings of the National Academy of Sciences of the USA* **91**, 10384–10388.

CLEVES, A. E., COOPER, D. N. W., BARONDES, S. H. & KELLY, R. B. (1996). A new pathway for protein export in *Saccharomyces cerevisiae*. *Journal of Cell Biology* **133**, 1017–1026.

COMB, M., SEEBURG, P. H., ADELMAN, J., EIDEN, L. & HERBERT, E. (1982). Primary structure of the human Met- and Leu-enkephalin precursor and its mRNA. *Nature* **295**, 663–666.

CONSTAM, D. B., CALFON, M. & ROBERTSON, E. (1996). SPC4, SPC6, and the novel protease SPC7 are coexpressed with bone morphogenetic proteins at distinct sites during embryogenesis. *Journal of Cell Biology* **134**, 181–191.

COOL, D. R., FENGER, M., SNELL, C. R. & LOH, Y. P. (1995). Identification of the sorting signal motif within pro-opiomelanocortin for the regulated secretory pathway. *Journal of Biological Chemistry* **270**, 8723–8729.

COOL, D. R., NORMANT, E., SHEN, F.-S., CHEN, H.-C., PANNELL, L., ZHANG, Y. & LOH, Y. P. (1997). Carboxypeptidase E is a regulated secretory pathway sorting receptor: genetic obliteration leads to endocrine disorders in Cpe^{fat} mice. *Cell* **88**, 73–83.

CORTI, O., DIDONATO, S. & FINOCCHIARO, G. (1994). Divergent sequences in the 5′ region of cDNA suggest alternative splicing as a mechanism for the generation of carnitine acteyltransferases with different subcellular locations. *Biochemical Journal* **303**, 37–41.

CREEMERS, J. W., KORMELINK, P. J., ROEBROEK, A. J., NAKAYAMA, K. & VAN DE VEN, W. J. (1993). Proprotein processing activity and cleavage site selectivity of the kex2-like endoprotease PACE4. *FEBS Letters* **336**, 65–69.

DANPURE, C. J. (1995). How can the products of a single gene be localized to more than one subcellular compartment? *Trends in Cell Biology* **5**, 230–238.

DICKINSON, C. J., SAWADA, M., GUO, Y. J., FINNISS, S. & YAMADA, T. (1995). Specificity of prohormone convertase endoproteolysis of progastrin in AtT20 cells. *Journal of Clinical Investigation* **96**, 1425–1431.

DUBOIS, C. M., LAPRISE, M. H., BLANCHETTE, F., GENTRY, L. E. & LEDUC, R. (1995). Processing of transforming growth factor β1 precursor by furin convertase. *Journal of Biological Chemistry* **270**, 10618–10624.

DUPUY, A., LINDBERG, I., ZHOU, Y., AKIL, H., LAZURE, C., CHRETIEN, M., SEIDAH, N. G. & DAY, R. (1994). Processing of prodynorphin by the prohormone convertase PC1 results in high molecular weight intermediate forms: cleavage at a single arginine residue. *FEBS Letters* **337**, 60–65.

DUVERGER, E., PELLERIN-MENDES, C., MAYER, R., ROCHE, A. C. & MONSIGNY, M. (1995). Nuclear import of glyconjugates is distinct from the classical NLS pathway. *Journal of Cell Biology* **108**, 1325–1332.

EGEL-MITANI, M., FLYGENRING, H. P. & HANSEN, M. T. (1990). A novel aspartyl protease allowing kex2-independent MF alpha propheromone processing in yeast. *Yeast* **6**, 127–137.

ESTIVARIZ, F. E., CARINO, M., LOWRY, P. J. & JACKSON, S. (1988). Further evidence that N-terminal pro-opiomelanocortin peptides are involved in adrenal mitogenesis. *Journal of Endocrinology* **116**, 201–206.

FLORKIEWICZ, R. Z., MAJACK, R. A., BUECHLER, R. D. & FLORKIEWICZ, E. (1995). Quantitative export of FGF-2 occurs through an alternative, energy-dependent, non-ER/Golgi pathway. *Journal of Cellular Physiology* **162**, 388–399.

FUMAGALLI, G. & ZANINI, A. (1985). In cow anterior pituitary, growth hormone and prolactin can be packed in separate granules of the same cell. *Journal of Biological Chemistry* **100**, 2019–2024.

GISS, B. J. & WALKER, A. M. (1985). Mammotroph autoregulation: intracellular fate of internalized prolactin. *Molecular and Cellular Endocrinology* **42**, 259–267.

GORR, S. V. & DARLING, D. S. (1995). An N-terminal hydrophobic peak is the sorting signal of regulated secretory proteins. *FEBS Letters* **361**, 8–12.

GORR, S. V., HAMILTON, J. W. & CHON, D. V. (1992). Regulated, but not constitutive, secretory proteins bind porcine chymotrypsinogen. *Journal of Biological Chemistry* **267**, 21595–21600.

GOURDJI, D., TIXIER-VIDAL, A., MORIN, A., PRADELLES, P., MORGAT, J. L., FROMAGEOT, P. & KERDELHUE, B. (1973). Binding of a tritiated thyrotropin-releasing factor to a prolactin secreting clonal cell line (GH3). *Experimental Cell Research* **82**, 39–46.

GUMBINER, B. & KELLY, R. B. (1982). Two distinct intracellular pathways transport secretory and membrane glycoproteins to the surface of pituitary tumour cells. *Cell* **28**, 51–59.

GUTTMAN, S. & BOISSONNAS, R. A. (1961). Influence of the structure of the N-terminal extremity of α-MSH on the melanophore stimulating activity of this hormone. *Experientia* **17**, 265–267.

HENDY, G. N., BENNETT, H. P., GIBBS, B. F., LAZURE, C., DAY, R. & SEIDAH, N. G. (1995). Proparathyroid hormone is preferentially cleaved to parathyroid hormone by the prohormone convertase furin. A mass spectrometric study. *Journal of Biological Chemistry* **270**, 9517–9525.

HIWASA, T. & SAKIYAMA, S. (1996). Nuclear localization of procathepsin L/MEP in ras-transformed mouse fibroblast. *Cancer Letters* **99**, 87–91.

HOPKINS, C. R. (1994). Internalisation of polypeptide growth factor receptors and the regulation of transcription. *Biochemical Pharmacology* **47**, 151–154.

HUANG, J. & PHILBERT, M. A. (1995). Distribution of glutathione and glutathione-related enzyme systems in mitochondria and cytosol of cultured cerebellar astrocytes and granule cells. *Brain Research* **680**, 16–22.

HURTLEY, S. M., BOLE, D. G., HOOVER-LITTY, H., HELENIUS, A. & COPELAND, C. S. (1989). Interactions of misfolded influenza virus hemagglutinin with binding protein (BiP). *Journal of Cell Biology* **108**, 2117–2126.

HYODO, S., YAMADA, C., TAKEZAWA, T. & URANO, A. (1992). Expression of provasopressin gene during ontogeny in the hypothalamus of developing mice. *Neuroscience* **46**, 241–250.

IMAMURA, T., ENGLEKA, K., ZHAN, X., TOKITA, Y., FOROUGH, R., ROEDER, D., JACKSON, A., MAIER, J. A. M., HLA, T. & MACIAG, T. (1990). Recovery of mitogenic activity of a growth factor mutant with a nuclear translocation sequence. *Science* **249**, 1567–1570.

ISHIKAWA, F., MIYAZONO, F., HELLMAN, U., DREXLER, H., WERSTEDT, C., HAGIWARA, K., USUKI, K., TAKAKU, F., RISAU, W. & HELDIN, C.-H. (1989). Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. *Nature* **338**, 557–562.

ITOH, N., OBATA, K., YANAIHARA, N. & OKAMOTO, H. (1983). Human intestinal polypeptide contains a novel PHI-27-like peptide, PHM-27. *Nature* **304**, 547–549.

JAYE, M., HOWK, R., BURGESS, W., RICCA, G. A., CHIER, I., RAVERA, M. W., O'BRIEN, S. J., MODI, W. S., MACIAG, T. & DROHAN, W. N. (1986). Human endothelial cell growth factor: Cloning, nucleotide sequence and chromosome localization. *Science* **233**, 541–545.

JENSEN, R. A., THOMPSON, M. E., JETTON, T. L., SZABO, C. I., VAN DE MEER, R., HELOU, B., TRONICH, S. R., PAGE, D. L., KING, M. C. & HOLT, J. T. (1996). BRCA 1 is secreted and exhibits properties of a granin. *Nature Genetics* **12**, 303–308.

JOHANSEN, T. E., SCHIFTER, S., VOGEL, C. K., TOLSTOY, S. & SCHWARTZ, T. W. (1991). Processing of pro-CGRP in a rat medullary thyroid carcinoma cell line transfected with protease inhibitors. *Molecular and Cellular Endocrinology* **82**, 51–60.

JULIUS, D., BRAKE, A., BLAIR, L., KUNISAWA, R. & THORNER, J. (1984). Isolation of the putative structural gene for the lysine–arginine-cleaving endopeptidase required for processing of yeast prepro-α-factor. *Cell* **37**, 1075–1089.

JUNG, L. J., KREINER, T. & SCHELLER, R. H. (1993). Expression of mutant ELH prohormones in AtT20 cells: the relationship between prohormone processing and sorting. *Journal of Cell Biology* **121**, 11–21.

KALDERON, D., RICHARDSON, W. D., MARKHAM, A. F. & SMITH, A. E. (1984). Sequence requirements for nuclear location of simian virus 40 large T-antigen. *Nature* **311**, 33–38.

KIEFER, P., ACLAND, P., PAPPIN, D., PETERS, G. & DICKSON, C. (1994). Competition between nuclear localization and secretory signals determines the subcellular fate of a single CUG-initiated form of FGF-3. *EMBO Journal* **13**, 4126–4136.

KIEFER, P. & DICKSON, C. (1995). Nucleolar association of fibroblast growth factor 3 via specific sequence motifs has inhibitory effects on cell growth. *Molecular and Cellular Biology* **15**, 4364–4374.

KUCHLER, K. & THORNER, J. (1990). Membrane translocation of proteins without hydrophobic signal peptides. *Current Opinion in Cell Biology* **2**, 617–624.

KWIATKOWSKI, D. J., MEHL, R. & YIN, H. L. (1988). Genomic organisation and biosynthesis of secreted and cytoplasmic forms of gelsolin. *Journal of Cell Biology* **106**, 375–384.

LADENHEIM, R. G., SEIDAH, N. G. & ROUGEON, F. (1991). N-linked glycosylation affects the processing of mouse submaxillary gland prorenin in transfected AtT20 cells. *European Journal of Biochemistry* **198**, 535–540.

LIN, L.-F. H., MISMER, D., LILE, J. D., ARMES, G., BUTLER, E. T., VANNICE, J. L. & COLLINS, F. (1990). FDC-P1 myeloid cells engineered to express fibroblast growth factor receptor 1 proliferate and differentiate in the presence of fibroblast growth factor and heparin. *Proceedings of the National Academy of Sciences of the USA* **89**, 3315–3319.

LIN, Y.-Z., YAO, S. Y. & HAWIGER, J. (1996). Role of the nuclear localization sequence in fibroblast growth factor-1-stimulated mitogenic pathways. *Journal of Biological Chemistry* **271**, 5305–5308.

LINDBERG, I., SHAW, E., FINLEY, J., LEONE, D. & DEININGER, P. (1991). Posttranslational modifications of rat proenkephalin overexpressed in chinese hamster ovary cells. *Endocrinology* **128**, 1849–1856.

LOBIE, P. E., MERTANI, H., MOREL, G., MORALES-BUSTOS, O., NORSTEDEDT, G. & WATERS, M. J. (1994). Receptor-mediated nuclear translocation of growth hormone. *Journal of Biological Chemistry* **269**, 21330–21339.

LOWENSTEIN, P. R., MORRISON, E. E., BAIN, D., SHERING, A. F., BANTING, G., DOUGLAS, P. & CASTRO, M. G. (1994). Polarized distribution of the trans-golgi network marker TGN38 during the *in vitro* development of neocortical neurons: effects of nocodazole and brefeldin A. *European Journal of Neuroscience* **6**, 1453–1465.

MACDONALD, H. S., KUSHNARYOV, V. M., SEDMARK, J. J. & GROSSBERG, S. E. (1986). Transport of χ-interferon into the cell nucleus may be mediated by nuclear membrane receptors. *Biochemical and Biophysical Research Communications* **138**, 254–260.

MARCH, C. J., MOSLELY, B., LARSEN, A., CERRETI, D. P., BRAEDT, G., PRICE, V., GILLIS, S., HENNEY, C. S., KRONHEIM, S. R., GRABSTEIN, K., CONLON, P. J., HOPP, P. T. & COSMAN, D. (1985). Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature* **315**, 641–647.

MARTENS, G. J. M., BRAKS, J. A. M., EIB, D. W., ZHOU, Y. & LINDBERG, I. (1994). The neuroendocrine polypeptide 7B2 is an endogenous inhibitor of prohormone convertase PC2. *Proceedings of the National Academy of Sciences of the USA* **91**, 5784–5787.

MERIGHI, A., POLAK, J. M., GIBSON, S. J., GULBENKIAN, S., VALENTINO, K. L. & PEIRONE, S. M. (1988). Ultrastructural studies on calcitonin gene-related peptide-, tachykinins- and somatostatin-immunoreactive neurones in rat dorsal root ganglia: Evidence for the colocalization of different peptides in single secretory granules. *Cell and Tissue Research* **254**, 101–109.

MERTANI, H. C., WATERS, M. J. & MOREL, G. (1996). Cellular trafficking of exogenous growth hormone in dwarf rat pituitary. *Neuroendocrinology* **63**, 257–268.

MILLAR, R. P., ROSEN, H., BADMINTON, M., PASQUALINI, C. & KERDELHUE, B. (1983). Luteinizing hormone-releasing hormone (LH-RH) binding to purified rat pituitary nuclei. *FEBS Letters* **153**, 382–386.

MOREL, G. (1994). Internalization and nuclear localization of peptide hormones. *Biochemical Pharmacology* **47**, 63–76.

MOREL, G., AUBERT, M. L. & DUBOIS, P. M. (1994). Intracellular gonadotropin-releasing hormone immunoreactivity in gonadotroph of intact or castrated male rats: semiquantitative estimation of testosterone and GnRH antagonist treatment. *Acta Anatomica* **149**, 46–54.

MORRISON, E., TOMASEC, P., LINTON, E. A., LOWRY, P. J., LOWENSTEIN, P. R. & CASTRO, M. G. (1995). Expression of biologically active procorticotrophin-releasing hormone (proCRH) in stably transfected CHO-K1 cells: characterization of nuclear proCRH. *Journal of Neuroendocrinology* **7**, 263–272.

NAKANISHI, S., INOUE, A., KITA, T., NAKAMURA, M., CHANG, A. C., COHEN, S. N. & NUMA, S. (1979). Nucleotide sequence of cloned cDNA for bovine corticotropin-β-lipotropin precursor. *Nature* **278**, 423–427.

NAWA, H., KOTANI, H. & NAKANISHI, S. (1984). Tissue-specific generation of two preprotachykinin mRNAs from one gene by alternative RNA splicing. *Nature* **312**, 729–734.

NEVES, F. A., DUNCAN, K. G. & BAXTER, J. B. (1996). Cathepsin B is a prorenin processing enzyme. *Hypertension* **27**, 514–517.

NOEL, G., KEUTMANN, H. T. & MAINS, R. E. (1991). Investigation of the structural requirements for peptide precursor processing in AtT20 cells using site-directed mutagenesis of proadrenocorticotropin/endorphin. *Molecular Endocrinology* **5**, 404–413.

NOVICK, P. & BOTSTEIN, D. (1985). Phenotypic analysis of temperature-sensitive yeast actin mutants. *Cell* **40**, 405–416.

OLDFINE, I. D., JONES, A. L., HRADEK, G. T., WONG, K. Y. & MOONEY, J. S. (1978). Entry of insulin into human cultured lymphocytes: electron microscope autoradiographic analysis. *Science* **202**, 760–763.

ORCI, L., RAVAZZOLA, M., AMHERDT, M., PERRELET, A., POWELL, S. K., QUINN, D. L. & MOORE, H.-P. H. (1987). The trans-most cisternae of the golgi complex: a compartment for sorting of secretory and plasma membrane proteins. *Cell* **51**, 1039–1051.

PATRY, V., ARNAUD, E., AMALRIC, F. & PRATS, H. (1994). Involvement of basic fibroblast growth factor NH_2 terminus in nuclear accumulation. *Growth Factors* **11**, 163–174.

PERONE, M. J., AHMED, I., LINTON, E. & CASTRO, M. G. (1996). Procorticotrophin releasing hormone is endoproteolytically processed by the prohormone convertase PC2 but not by PC1 within stably transfected CHO-K1 cells. *Biochemical Society Transactions* **24**, 497S.

POULAT, F., GIRARD, F., CHEVRON, M. P., GOZE, C., REBILLARD, X., CALAS, B., LAMB, N. & BERTA, P. (1995). Nuclear localization of the testis determining gene product SRY. *Journal of Cell Biology* **128**, 737–748.

RAJENDRAN, K. G. & MENON, K. M. J. (1983). Evidence for the existence of gonadotropin receptors in the nuclei isolated from rat ovary. *Biochemical and Biophysical Research Communications* **111**, 127–134.

RAKWICZ-SZULCZYNSKA, E. M., RODECK, U., HERLYN, M. & KOPROWSKI, H. (1986). Chromatin binding of epidermal growth factor, nerve growth factor and platelet-derived growth factor in cells bearing the appropriate surface receptors. *Proceedings of the National Academy of Sciences of the USA* **83**, 3728–3732.

RAO, C. V. & MITRA, S. (1979). Gonadotropin and prostaglandins binding sites in nuclei of bovine corpora lutea. *Biochimica et Biophysica Acta* **584**, 454–466.

RE, R. N., VIZARD, D. L., BROWN, J. & BRYAN, S. E. (1984). Angiotensin II receptors in chromatin fragments generated by micrococcal nuclease. *Biochemical and Biophysical Research Communications* **119**, 220–227.

REDDY, K. B., JIN, G., KARODE, M. C., HARMONY, J. A. & HOWE, P. H. (1996). Transforming growth factor beta (TGF beta)-induced nuclear localization of apolipoprotein J/clusterin in epithelial cells. *Biochemistry* **35**, 6157–6163.

ROBBINS, J., DILWORTH, S. M., LASKEY, R. A. & DINGWALL, C. (1991). Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. *Cell* **64**, 615–623.

ROTHENBERG, M. E., EILERTSON, C. D., KLEIN, K., ZHOU, Y., LINDBERG, I., MCDONALD, J. K., MACKIN, R. B. & NOE, B. (1995). Processing of mouse proglucagon by recombinant prohormone convertase 1 and immunopurified prohormone convertase 2 in vitro. *Journal of Biological Chemistry* **270**, 10136–10146.

ROUILLE, Y., WESTERMARK, G., MARTIN, S. K. & STEINER, D. F. (1994). Proglucagon is processed to glucagon by prohormone convertase PC2 in α-TC1-6 cells. *Proceedings of the National Academy of Sciences of the USA* **91**, 3242–3246.

RUBARTELLI, A., BAJETTO, A., ALLAVENA, G., WOLLMAN, E. & SATIA, R. (1992). Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. *Journal of Biological Chemistry* **267**, 24161–24164.

RUBARTELLI, A., COZZOLINO, F., TALIO, M. & SITIA, R. (1990). A novel secretory pathway for interleukin-1β, a protein lacking a signal sequence. *EMBO Journal* **9**, 1503–1510.

SARIA, A., GAMSE, R., PETERMANN, J., FISCHER, J. A., THEODORSSON-NORHEIM, E. & LUNDBERG, J. M. (1986). Simultaneous release of several tachykinins and calcitonin gene-related peptide from rat spinal cord slices. *Neuroscience Letters* **63**, 310–314.

SAVION, N., VLODAVSKY, I. & GOSPODAROWICZ, D. (1981). Nuclear accumulation of epidermal growth factor in cultured bovine corneal endothelial and granulosa cells. *Journal of Biological Chemistry* **256**, 1149–1154.

SCHILLER, M. R., KHON, A. B., MENDE-MUELLER, L. M., MILLER, K. & HOOK, V. Y. H. (1996). Expression of recombinant pro-neuropeptide Y, proopiomelanocortin, and proenkephalin: relative processing by prohormone thiol protease (PTP). *FEBS Letters* **382**, 6–10.

SCHMIDT, W. K. & MOORE, H.-P. H. (1994). Synthesis and targeting of insulin-like growth factor-I to the hormone storage granules in an endocrine cell line. *Journal of Biological Chemistry* **269**, 27115–27124.

SEIDAH, N. G., BENJANNET, S., PAREEK, S., SAVARIA, D., HAMELIN, J., GOULET, B., LALIBERTÉ, J., LAZURE, C., CHRETEN, M. & MURPHY, R. A. (1996). Cellular processing of the nerve growth factor precursor by the mammalian pro-protein convertases. *Biochemical Journal* **314**, 951–960.

SEIDAH, N. G., CHRETIEN, M. & DAY, R. (1994). The family of subtilisin/kexin like pro-protein and pro-hormone convertases: Divergent or shared functions. *Biochimie* **76**, 197–209.

SEVARINO, K. A., STORK, P., VENTIMIGLIA, R., MANDEL, G. & GOODMAN, R. H. (1989). Amino-terminal sequences of prosomatostatin direct intracellular targeting but not processing specificity. *Cell* **57**, 11–19.

SHENNAN, K. I. J., TAYLOR, N. A., JERMANY, J. L., MATTHEWS, G. & DOCHERTY, K. (1995). Differences in pH optima and calcium requirements for maturation of the prohormone convertases PC2 and PC3 indicates different intracellular locations for these events. *Journal of Biological Chemistry* **270**, 1402–1407.

SHIURBA, R. A., JING, N., SAKAKURA, T. & GODSAVE, S. F. (1991). Nuclear translocation of fibroblast growth factor during *Xenopus* mesoderm induction. *Development* **113**, 487–493.

SKOFITSCH, G. & JACOBOWITZ, D. M. (1985). Calcitonin gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. *Peptides* **6**, 747–754.

SMEEKENS, S. P. (1993). Processing of protein precursors by a novel family of subtilisin-related mammalian endoproteases. *Bio/Technology* **11**, 182–186.

SMEEKENS, S. P., ALBIGES-RIZO, C., CARROLL, R., MARTIN, S., OHAGI, S., PHILLIPS, L. A., BENIG, M., GARDNER, P., MONTAG, A. G., SWIFT, H. H., THOMAS, G. & STEINER, D. F. (1992). Proinsulin processing by the subtilisin-related pro-protein convertases furin, PC2 and PC3. *Proceedings of the National Academy of Sciences of the USA* **89**, 8822–8827.

SMITH, R. M. & JARETT, L. (1990). Partial characterization of mechanism of insulin accumulation in H35 hepatome cell nuclei. *Diabetes* **39**, 683–689.

SOLARI, R., SMITHERS, N., KENNARD, N., RAY, K. & GRENFELL, S. (1994). Receptor mediated endocytosis and intracellular fate of interleukin 1. *Biochemical Pharmacology* **47**, 93–101.

SOSSIN, W. S., SWEET-CORDERO, A. & SCHELLER, R. H. (1990). Dale's hypothesis revisited: different neuropeptides derived from a common prohormone are targeted to different processes. *Proceedings of the National Academy of Sciences of the USA* **87**, 4845–4848.

SPENCE, A. M., SHEPPARD, P. C., DAVIE, J. R., MATUO, Y., NICHI, N., MCKEEHAN, W. L., DODD, J. G. & MATUSIK, R. J. (1989). Regulation of a bifunctional mRNA results in synthesis of secreted and nuclear probasin. *Proceedings of the National Academy of Sciences of the USA* **86**, 7843–7847.

SRINIVASAN, M., EDMAN, C. F. & SCHULMAN, H. (1994). Alternative splicing introduces a nuclear localisation signal that targets multifunctional CaM kinase to the nucleus. *Journal of Cell Biology* **126**, 839–852.

TAUSSIG, R. & SCHELLER, R. H. (1986). The *Aplysia* FMRFamide gene encodes sequences related to mammalian brain peptides. *DNA* **5**, 453–461.

TOOZE, J., KERN, H. F., FULLER, S. D. & HOWELL, K. E. (1989). Condensation-sorting events in the rough endoplasmic reticulum of exocrine pancreatic cells. *Journal of Cell Biology* **109**, 35–50.

TOOZE, S. A. & HUTTNER, W. B. (1990). Cell-free protein sorting to the regulated and constitutive secretory pathways. *Cell* **60**, 837–847.

VAN DE VEN, W. J. M., VOORBERG, J., FONTIN, R., PANNEKOEK, H., ANS, M. W., VAN DEN OUWELAND, A. M. V., DUIJNHOVEN, H. L. P., ROEBROEK, A. J. M. & SIEZEN, R. J. (1990). Furin is a subtilisin-like proprotein processing enzyme in higher eukaryotes. *Molecular Biology Reports* **14**, 265–275.

WANG, T. C., KOH, T. J., VARRO, A., CAHILL, R. J., DANGLER, C. A., FOX, J. G. & DOCKRAY, G. J. (1996). Processing and proliferative effects of human progastrin in transgenic mice. *Journal of Clinical Investigation* **98**, 1918–1929.

WULFF, B. S., JOHANSEN, T. E., DALBOGE, H., O'HARE, M. M. T. & SCHWARTZ, T. W. (1993). Processing of two homologous precursors, pro-neuropeptide Y and pro-pancreatic polypeptide, in transfected cell lines expressing different precursor convertases. *Journal of Biological Chemistry* **268**, 13327–13335.

YANKER, B. A. & SHOOTER, E. M. (1979). Nerve growth factor in nucleus: interaction with receptor on the nuclear membrane. *Proceedings of the National Academy of Sciences of the USA* **76**, 1269–1273.

YOON, J. Y. & BEINFELD, M. C. (1995). Expression of antisense PC1 in stably transfected RIN5F cells significantly reduces CCK 8 biosynthesis. *Regulatory Peptides* **59**, 221–227.

ZOLMAN, J. C. & THEODOROPOULOS, T. J. (1984). Localization of putative gonadotrophin releasing hormone receptor protein in the anterior pituitary. *Cell Biochemistry and Function* **2**, 208–212.