REVIEW ARTICLE

Neuroendocrine secretory protein 7B2: structure, expression and functions

Majambu MBIKAY*[1], Nabil G. SEIDAH† and Michel CHRÉTIEN*

*Diseases of Aging Program, Ottawa Health Research Institute, Ottawa Hospital, University of Ottawa, Ottawa, Ontario, Canada Y1K 4K9, and †Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, University of Montreal, Montreal, Quebec, Canada H2W 1R7

7B2 is an acidic protein residing in the secretory granules of neuroendocrine cells. Its sequence has been elucidated in many phyla and species. It shows high similarity among mammals. A Pro-Pro-Asn-Pro-Cys-Pro polyproline motif is its most conserved feature, being carried by both vertebrate and invertebrate sequences. It is biosynthesized as a precursor protein that is cleaved into an N-terminal fragment and a C-terminal peptide. In neuroendocrine cells, 7B2 functions as a specific chaperone for the proprotein convertase (PC) 2. Through the sequence around its Pro-Pro-Asn-Pro-Cys-Pro motif, it binds to an inactive proPC2 and facilitates its transport from the endoplasmic reticulum to later compartments of the secretory pathway where the zymogen is proteolytically matured and activated. Its C-terminal peptide can inhibit PC2 *in vitro* and may contribute to keep the enzyme transiently inactive *in vivo*. The PC2–7B2 model defines a new neuroendocrine paradigm whereby proteolytic activation of prohormones and proneuropeptides in the secretory pathway is spatially and temporally regulated by the dynamics of interactions between converting enzymes and their binding proteins. Interestingly, unlike PC2-null mice, which are viable, 7B2-null mutants die early in life from Cushing's disease due to corticotropin ('ACTH') hypersecretion by the neurointermediate lobe, suggesting a possible involvement of 7B2 in secretory granule formation and in secretion regulation. The mechanism of this regulation is yet to be elucidated. 7B2 has been shown to be a good marker of several neuroendocrine cell dysfunctions in humans. The possibility that anomalies in its structure and expression could be aetiological causes of some of these dysfunctions warrants investigation.

Key words: chaperone, proprotein convertase, proteolytic processing.

INTRODUCTION

Limited proteolysis of precursor polypeptides is now recognized as a common biological mechanism for the production of bioactive proteins and peptides [1]. Neuroendocrine cells manufacture peptide hormones and neuropeptides through such a mechanism. The proprotein convertases (PCs) 1/3 and 2 are the principal mediators of prohormone and proneuropeptide processing in the secretory pathway of these cells [2,3]. PC1/3 and PC2 are themselves biosynthesized in the endoplasmic reticulum (ER) as inactive precursor proteins that are transported through the Golgi and the *trans*-Golgi network (TGN) into secretory granules, and are proteolytically activated along the way. The timing of this activation is a major determinant in the coordinated processing of prohormones and proneuropeptides. Thus in pituitary corticotrophs, PC1/3 mediates the initial processing of pro-opiomelanocortin (POMC) into corticotropin ('ACTH') and β-lipotropic hormone (β-LPH), and PC2 converts β-LPH into β-melanocyte-stimulating hormone and β-endorphin [4]. The autocatalytic conversion of proPC1/3 occurs in the ER and its activation takes place in the TGN and immature granules through secondary cleavages within the pro-segment and the C-terminal domain. Comparatively, proPC2 maturation into PC2 and the subsequent activation of the latter are delayed until the zymogen has reached the post-Golgi compartments [5]. Most neuroendocrine cells contain 7B2, an acidic protein of approx. 150 residues, whose cellular function has long remained unknown. It was discovered in recent years that this protein binds PC2 and facilitates its transport and activation, thus defining an additional level of control of converting activities in neuroendocrine cells. This article reviews the biology of 7B2, from its structure to its function.

THE DISCOVERY OF 7B2

7B2 was discovered in 1982 by Seidah, Chrétien and their collaborators, while purifying the N-terminal glyco-segment of POMC from pig anterior pituitaries [6]. It eluted under a distinct peak in a reverse-phase HPLC profile of tissue extracts. The sequence of its first 50 amino acids showed a weak identity with duck proinsulin (30%) and pig secretin (26%) [6]. Soon after, the same investigators reported the purification of its human pituitary homologue, as well as the 77-residue N-terminal sequences for both the human and porcine proteins [7]. The two sequences differed by only one residue. This high evolutionary conservation suggested that 7B2 might be biologically relevant. The purified protein migrated with a molecular mass of 19–21 kDa, as determined by SDS/PAGE, and with a pI of 4.9 in an isoelectric focusing gel [6–8]. However, by gel permeation chromatography of tissue extracts, 7B2 immunoreactivity eluted with varying molecular masses, ranging from 11 to 50 kDa [8–10], an early indication that the protein might be processed and could associate with other proteins.

Abbreviations used: AP, activator protein; 7B2CT, C-terminal domain of 7B2; CPE, carboxypeptidase E; CRE, cAMP-responsive element; CRF, corticotropin-releasing factor; DTT, dithiothreitol; ER, endoplasmic reticulum; GH, growth hormone; IBMX, 3-isobutyl-1-methyl-xanthine; IGF, insulin-like growth factor; LHRH, luteinizing hormone-releasing hormone; β-LPH, β-lipotropic hormone; PACE, paired basic amino acid converting enzyme; PC, proprotein convertase; POMC, pro-opiomelanocortin; PWS, Prader–Willi syndrome; SH3, Src homology 3; TGN, *trans*-Golgi network; UTR, untranslated region; VP, vasopressin.

[1] To whom correspondence should be addressed (e-mail mmbikay@ohri.ca).

7B2 PROTEIN AND GENE STRUCTURES

Over the years, the complete sequence of 7B2 was deduced from the sequence of cDNAs cloned in many species, organs and cell lines, including human anterior pituitary [11], mouse insulinoma [12], toad intermediate pituitary [13], rat insulinoma [14], pig adrenal medulla [15], fish hypothalamus [14], mollusc brain [16], nematode [17] and fruit fly [18]. An alignment between these sequences is shown in Figure 1. The overall residue identity is very high (90–96 %) among mammals, relatively high (67–83 %) between mammals and frog or fish, and low (17–22 %) between vertebrates and invertebrates. The most conserved feature among all 7B2 sequences is a Pro-Pro-Asn-Pro-Cys-Pro motif corresponding to residues 90–95 in human 7B2 (hereinafter we will refer to the numbering of human 7B2 in Figure 1 to identify residues and domains). Among mammalian 7B2s, other conserved structural features include: clusters of basic residues (residues 139–140, 151–155 and 172–173), representing potential sites of processing by serine endoproteinases of the PC family [2,3]; a serine phosphorylation consensus site (Ser^{174}); and a Glu- and Asp-rich acidic C-terminus (residues 180–186).

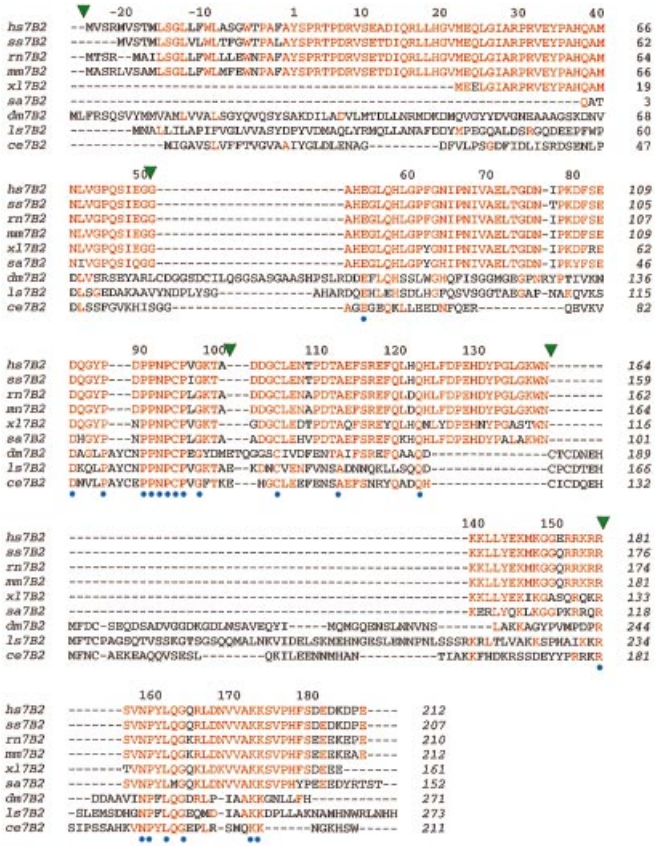

Figure 1 Alignment of known 7B2 sequences

The sequences were deduced from GenBank®-retrieved or published (see the text) cDNA sequences. The species (the scientific name, prefix and GenBank® accession numbers) are: man (*Homo sapiens*, *hs*, NM 003020); pig (*Sus scrofa*, *ss*, M23654); rat (*Rattus norvegicus*, *rn*, NM 13175); mouse (*Mus musculus*, *mm*, NM 009162); toad (*Xenopus laevis*, *xl*, X15608), salmon (*Salmo salar*, *sa*); fruit fly (*Drosophila melanogaster*, *dm*, AJ271974); snail (*Lymneae stagnalis*, *ls*, U72702); and worm (*Caenorhabditis elegans*, *ce*). The human 7B2 sequence is numbered every ten residues on top of the aligned sequences, starting with the first residue after its signal peptide. For all sequences, the number of the terminal residue in each line is given to the right. Residues identical among all 7B2s are highlighted with blue dots. Those identical among or with mammalian sequences are highlighted in red. The genetic sequence encoding the human 7B2 is interrupted by introns at positions indicated with green arrowheads.

Interestingly, after the signal peptide, the only discontinuity among mammalian 7B2 sequences is the presence or the absence of an alanine residue following Thr^{99} (Figure 1). Southern-blot analysis of cDNA clones randomly retrieved from a cDNA library with isoform-specific 7B2 oligonucleotide probes showed that five out of seven of these clones lacked the Ala^{100} codon [19]. Their coexistence in other endocrine tissues and in different species was suggested by heteroduplex analysis of DNA amplicons by reverse transcriptase-PCR of their RNA [19]. The alanine residue was absent in the cDNA-deduced porcine 7B2 sequence [14], but was present in the amino acid sequence of the 7B2 protein purified from pig pituitary extracts [20]. A variety of models can be proposed to explain the presence of Ala^{100} in the sequence of 7B2 protein purified from tissues and the prevalence of 7B2 transcripts without the Ala^{100} codon among cloned mRNAs. On one hand, there is the possibility of preferential utilization of the Ala^{100+}-encoding mRNA isoform for translation coupled or not with its selective degradation. On the other hand, it is possible that the Ala^{100-} protein may have a shorter half-life and may therefore be under-represented in tissue-extracted 7B2. There are naturally occurring precedents for all these possibilities. For example, differential translation has been observed between anglerfish prosomatostatin mRNA I and its isoform, II, which contains three octanucleotide repeats in its 5′-untranslated region (UTR) [21]. Similarly, among the three insulin-like growth factor (IGF) mRNA isoforms of 6, 5 and 4.8 kb, differing by their leader sequence, the two shortest are preferentially utilized relative to the more abundant 6 kb isoform [22]. Although the structure of the 5′-UTR may be determining the efficiency of initiation in these two examples, theoretical computation of the structure of $codon^{100+}$ and $codon^{100-}$ mRNA using the MFOLD program [23] produced distinctive stem-loop structures in the region encompassing this codon (results not shown). Such structural differences may influence the relative unwinding, translatability and stability of the two isoforms. Preferential utilization of the $codon^{100+}$-7B2 mRNA may be associated with its selective degradation. Recruitment for translation is known to render some mRNAs susceptible to degradation by nucleases. The degradation is initiated by endonucleases and completed by exonucleases. Its extent may be partly determined by stabilizing or destabilizing RNA-binding proteins that recognize specific stem-loop structures in the mRNA [24]. Differential stability at the protein level is illustrated by the $\Delta K60$ human $\alpha 1$ globin mutation in which a single codon deletion leads to the production of protein that lacks Lys^{60} and is rapidly degraded [25]. Whether any of these models is applicable to 7B2 isoforms is surely amenable to experimental verification by a combination of ribosome-loading and pulse–chase studies using isoform-specific probes and antibodies. It should also be noted that the variation is located within a structural loop generated by the single and functionally important (as discussed later) disulphide bridge of the molecule. In view of the conservation of these isoforms across mammalian species, it is conceivable that the single-residue difference may subtly influence the cellular functions of 7B2.

The structure of the 7B2 gene in rat and human is known [14,26,27]. The human gene is more than 30 kb long and is made of six exons (Figure 2). Exon 1 specifies the 5′-UTR of the mRNA, exon 2 specifies the signal peptide and residues 1–50 of pro7B2, exon 3 specifies residues 51–101, exon 4 specifies residues 102–138, exon 5 specifies residues 139–155, and exon 6 specifies residues 156–186 (see Figure 1). The presence or absence of Ala^{100} is probably due to alternative utilization of two different 3′ splice sites that are three nucleotides apart at the end of the

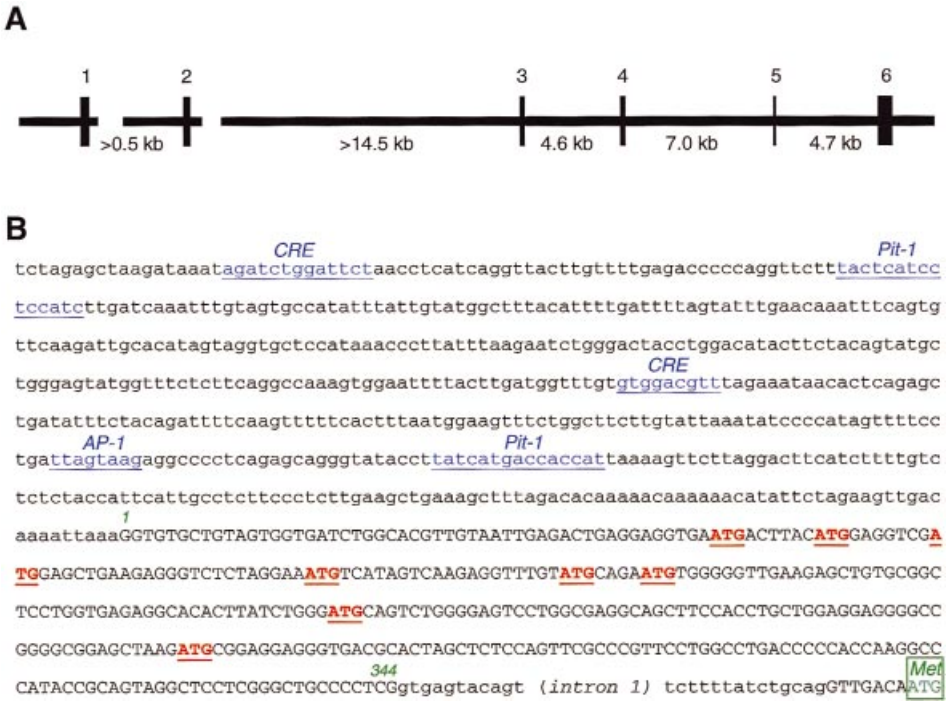

Figure 2 Structure and sequence of the promoter region of the human 7B2 gene

(**A**) Schematic representation of the gene based on GenBank® data (accession numbers: AC074201, AJ290437-41 and X94303). Exons are shown as vertical bars. The exact sizes of introns 1 and 2 have not been determined. (**B**) Sequence of the proximal promoter and the first exon. The exon sequence is shown in capital letters. Putative regulatory elements in the upstream sequence are underlined and the potential binding factors are indicated above in blue. Out-of-frame ATGs in the first exon are shown in bold red and are also underlined. The initiator ATG in the second exon is shown in green and boxed.

third intron [26]. Exons 4, 5 and 6 specify the two most important functional domains of 7B2 (see below). The human 7B2 locus (*SGNE1*) maps on human chromosome 15q [28]. In mouse, its homologue (*Sgne1*) maps on the E3-F3 region of chromosome 2 [26].

TISSUE DISTRIBUTION AND SUBCELLULAR LOCALIZATION OF 7B2

In addition to cloned cDNA probes, a number of polyclonal and monoclonal antibodies against defined domains of 7B2 have been raised [7,29–32]. This has permitted a detailed study of its tissue distribution, subcellular localization, biosynthesis and secretion. Table 1 describes the distribution of 7B2 in normal tissues as determined by various studies using radioimmunoassays, immunohistochemistry, immunocytochemistry or *in situ* hybridization. The mRNA or the protein are found in tissues that are either primarily neuronal (e.g. brain and adrenal medulla) or endocrine (e.g. pituitary, thyroid and pancreas), or are known to carry a sub-population of neuroendocrine cells (e.g. the gastrointestinal tract). In rat, in pg/mg of total proteins, the anterior lobe of the pituitary contains the highest amount of immunoreactive 7B2, followed by the neurointermediate lobe, the hypothalamus, the adrenal medulla, the thyroid gland and the pancreas. Other tissues contain relatively minor or undetectable amounts of the protein [8].

Within neuroendocrine cells, 7B2 is located inside secretory granules. This was initially determined by subcellular fractionation of porcine and rat neurointermediate lobes [8,33], and by immunocytochemistry on rat hypothalami and porcine chromaffin granules [10,33]. This localization was later confirmed for a variety of other neuroendocrine tissues and cells, where it often overlaps with that of resident hormones and neuropeptides (Table 2).

Table 1 Tissue distribution of 7B2

Abbreviations used: b, bovine; gp, guinea pig; H, human; ICC, immunocytochemistry; IHC, immunohistochemistry; ISH, *in situ* hybridization; p, pig; r, rat; RIA, radioimmunoassay; x, *Xenopus*.

Organs	Species	Methods	References
Brain	H, r	RIA, IHC, ISH	[9,34–36]
Hypothalamus	r	RIA, IHC, ISH	[8,34–37]
Supraoptic nucleus	r	IHC	[35,37]
Paraventricular nucleus	r	IHC	[35,37]
Median eminence	x	IHC, ISH	[38]
Basal ganglia	r	RIA	[34]
Spinal cord	r	RIA, IHC, ISH	[33,35,36,39]
Pituitary	r	RIA, IHC	[34,35]
Anterior lobe	r, x	RIA, IHC, ISH	[8,35,38]
Neurointermediate lobe	r, x	RIA, IHC, ISH	[8,38]
Thyroid	r	RIA	[8]
Adrenal medulla	b, r	ICC, RIA	[8,40]
Gastrointestinal tract			
Pancreas	H, gp, p, r	RIA	[8,41]
Antrum	H, gp, p, r	RIA, IHC	[41–43]
Fundus	r	IHC	[41,43]
Ileum	H, gp, p, r	RIA, IHC	[8,41–43]
Colon	H, gp, p, r	RIA, IHC	[41,42]
Duodenum	H, gp, p, r	IHC	[41,42]
Skin Merkel cells	p	IHC	[44]

Table 2 Co-localization of 7B2 with hormones and neuropeptides

Abbreviations used: c, cat, ch, chick; FSH, follicle-stimulating hormone; H, human; h, hamster; ICC, immunocytochemistry; IHC, immunohistochemistry; ISH, *in situ* hybridization; m, mouse; MSH, melanocyte-stimulating hormone; PP, pancreatic polypeptide; r, rat; RIA, radioimmunoassay; TSH, thyroid-stimulating hormone; x, *Xenopus*.

Organs	Resident proteins	Species	Methods	References
Hypothalamus				
Supraoptic nucleus	VP	r	IHC	[37]
Paraventricular nucleus	VP	r	IHC, IHC	[37,45]
	Galanin	r	IHC	[46]
	Oxytocin	r	IHC, ISH	[45]
	Neuromedin B or U	H, r, m	IHC	[47]
Pituitary				
Anterior lobe	LH	r	ISH, IHC	[48,49]
	FSH	r	ISH, IHC	[48,49]
	TSH	r	ISH, IHC	[48,49]
	Corticotropin	r	ISH	[49]
	GH	r	ISH	[49]
	Prolactin	r	ISH	[49]
Neurointermediate lobe	POMC	r, x	ISH, IHC	[49]
	MSH	r	ISH	[49]
Posterior lobe	VP	r	IHC	[37]
Thyroid parafollicular cells	Calcitonin	H	IHC, ICC	[10]
Lung neuroepithelial bodies	Calcitonin gene-related peptide	c, r, h	IHC	[50,51]
Adrenal medulla	Somatostatin	ch	IHC	[52]
	Met-enkephalin	ch	IHC	[52]
	Neuropeptide Y	ch	IHC	[52]
Gastrointestinal tract				
Pancreas	Insulin	r	IHC	[53]
	Glucagon	r	IHC	[53]
	Somatostatin	r	IHC	[53]
	PP	r	IHC	[53]

BIOSYNTHESIS AND SECRETION OF 7B2

The first pulse–chase studies to examine the biosynthesis of 7B2 were conducted by Ayoubi et al. [54] using *Xenopus laevis* neurointermediate lobes, and by Paquet et al. [55] using mammalian cell lines. These studies showed that 7B2 is produced as an intracellular precursor of 25–29 kDa that gets converted into a secreted form of 18–21 kDa. The protein is not glycosylated [54], but is tyrosine-sulphated [55]. Two products of these post-translational modifications have been independently isolated from cell and tissue extracts, and have been characterized by protein sequencing: a $7B2^{1–150}$ protein purified from porcine pituitaries [20], and a $7B2^{174–186}$ phosphorylated peptide from bovine chromaffin granules [56]. The former was presumably generated by PC cleavage after the Arg^{151}-Arg-Lys-Lys-Arg^{155} basic quintuplet followed by carboxypeptidase E (CPE) removal of the basic residues; the latter by cleavage after Lys^{172}-Lys^{173} (see Figure 1). In mammals, the PC most likely to be responsible for the endoproteolysis at the basic quintuplet is furin. This was determined by coexpressing pro7B2 and various PCs in mammalian cells using recombinant vaccinia viruses as vectors, and comparing the ability of these enzymes to process the precursor form of 7B2 to its smaller forms. Furin cleavage of pro7B2 occurs in the Golgi and requires Arg^{152} at the fourth residue before the cleavage site [55]. Other convertases, such as PC1, PC2, paired basic amino acid converting enzyme (PACE) 4-A, PC5 and PC7, can also cleave pro7B2 at this site *ex vivo* [57–59]. It is worth noting that in *Xenopus*, pro7B2 processing takes place after the Lys^{116}-Lys^{117} pair preceding the furin cleavage site [54] (see Figure 1). Processing at such a pair may also occur in *Drosophila* and *Lymneae* pro7B2s, which lack any furin recognition site (see Figure 1). The enzyme mediating cleavage at this alternative site has not been determined.

After processing, 7B2 proteins are packaged into dense-core vesicles and are secreted upon exocytotic stimulation. This secretion was initially studied using primary cultures of rat anterior pituitary cells, because of the relative abundance of the molecule in these cells [8,60]. It was stimulated by plasma membrane depolarization with potassium ions and by treatment with luteinizing hormone-releasing hormone (LHRH) [60,61]. It was reduced by treatment with testosterone [61], and was unaffected by treatment with corticotropin-releasing factor (CRF) and growth hormone (GH)-releasing factor ('GRF') [60]. These observations suggested that, among pituitary cell types, gonadotrophs contain the largest amount of stored 7B2. This was confirmed by immunohistochemistry [48]. Regulated secretion was also demonstrated with bovine chromaffin granules [62], rat phaeochromocytoma PC12 cells [63], and hamster pancreatic islets [64]. The packaging of 7B2 into secretory granules seems to depend on the integrity of its single disulphide bond, as suggested by its constitutive secretion by *Xenopus* intermediate pituitaries treated with dithiothreitol (DTT) [65].

REGULATION OF 7B2 EXPRESSION

7B2 gene expression is developmentally and hormonally regulated. It is induced in the mouse embryonic carcinoma cell line P19 following retinoic acid-promoted neuronal differentiation [66]. It is up-regulated by dexamethasone in the rat medullary thyroid carcinoma cell line rMTC [67], and by phorbol esters and forskolin in rat insulinoma and glucagonoma [14,68]. It is down-

regulated in rat adrenal medulla by adrenergic and cholinergic signals [69], and by light in toad intermediary pituitary [13].

The *cis* elements involved in this regulation have not been analysed. The sequence of the proximal promoter of the 7B2 gene is known in human and in rat [14,27]. It lacks obvious TATA and CAAT boxes, but contains several regulatory elements including binding sites for cAMP-responsive element (CRE) binding proteins, activator protein (AP) 1 and pituitary-specific transcription factor 1/growth hormone factor 1 ('Pit-1/GHF-1') (Figure 2B). The presence of multiple Pit-1/GHF-1 binding sites may contribute to the elevated expression of 7B2 in the pituitary. Uncharacterized elements located in the first intron of the rat gene appear to be important for the regulation of this gene by forskolin or phorbol esters [14,68].

7B2 expression may also be regulated at post-transcriptional levels. Temperature shift of the *Xenopus* intermediary lobes from 22 °C to 30 °C induces accumulation of the 18 kDa processed 7B2, but not of its mRNA, consistent with translational or post-translational regulation. In mouse corticotroph AtT-20 cells, exocytotic stimulation with CRF or KCl causes a 50–60% decrease in the level of 7B2 mRNA within 30 min, followed by a return to normal levels after 60 min (M. Mbikay, unpublished work). This observation suggests that degradation of its mRNA may be a mechanism of controlling the level of this protein in neuroendocrine cells. Translation regulation is also suggested by the intriguing case of the homozygous Brattleboro (*di*/*di*) rat. This rat suffers from severe diabetes insipidus due to a deficiency in vasopressin (VP) from the supraoptic and paraventricular nuclei of the hypothalamus. The VP gene of *di*/*di* rats carries a frameshift-causing single nucleotide deletion, which leads to the production of a C-terminally extended VP precursor that is retained in the ER. This extended precursor is the major form produced early in life. Later, however, some solitary neurons begin to produce a variant form of the prohormone from transcripts containing a dinucleotide deletion downstream of the original one. The variant prohormone can be transported through the secretory pathway and processed into VP. This leads to attenuation of the diabetes and a heterozygote-like phenotype. Interestingly, early in the life of *di*/*di* rats, 7B2 immunoreactivity is detectable in oxytocin neurons, but not in VP neurons [46], even though both neuronal types carry comparable levels of 7B2 mRNA [45]. With age, as the rats acquire a heterozygote-like phenotype, 7B2 immunoreactivity becomes detectable in some VP neurons too [46]. Blockage of pro7B2 biosynthesis due to the accumulation of the C-terminally extended VP precursor in shared ER compartments and competition between the two precursors for the translocation machinery have been suggested as possible explanations for this phenomenon [46,70].

Some sequence features of the 7B2 mRNA suggest that it may be a poor template for protein synthesis. Indeed, although moderately rich in G and C bases (58.57% in human 7B2), the 5′-UTR of 7B2 mRNAs is unusually long (> 300 nt, see Figure 2B). The average length of this region in mammalian mRNAs is approx. 80 nt. Long 5′-UTRs have been shown to form complex secondary structures that negatively affect translation initiation, by physical hindrance or through interaction with cytosolic regulatory proteins [71–73]. Secondary structure prediction using the MFOLD algorithm [23] indicates that the human 7B2 5′-UTR may form relatively stable stem-loop structures ($\Delta G = -126$ to -132 kcal/mol, where 1 cal ≡ 4.184 J), some of them located near the 5′ end of the UTR. Such structures are likely to impede ribosomal scanning at translation initiation [74]. Moreover, this UTR is burdened by multiple out-of-frame AUGs (see Figure 2B). Such AUGs are often observed in the 5′-UTRs of mRNAs for tightly controlled proteins, such as growth factors and oncogenes [75]. Because of these features, the 7B2 mRNA may not be efficiently utilized by the translation machinery when initiation factors are limiting. Differential translation may also explain why, out of the five cell types in the adenohypophysis, which all express 7B2 mRNA, only two appear to contain detectable levels of 7B2 protein [49]. The corollary to this hypothesis is that 7B2 production may be very sensitive to signals that affect the translational capacity of the cell, of the kind observed in glucose-stimulated pancreatic cells [76,77].

CELLULAR FUNCTIONS OF 7B2

Pro7B2 binds proPC2

A momentous breakthrough in the study of 7B2 came about in 1994 when Braks and Martens [78], led by structural similarities between the first 90-amino-acid sequence of 7B2 and a segment of human, wheat and *Escherichia coli* chaperonins, gathered experimental evidence showing that a recombinant 7B2, added to metabolically radiolabelled *Xenopus* pituitary extracts and immunoprecipitated, can retrieve the various forms of PC2 from these extracts. They also showed by pulse–chase studies that proPC2 is bound to pro7B2 in the early compartments of the secretory pathway and dissociates from it in later ones. They proposed that 7B2 serves as an intracellular proPC2 chaperone and prevents the premature activation of the zymogen during its transit in the regulated secretory pathway. The dynamic interaction between these two neuroendocrine molecules was further characterized in numerous other studies [65,79–86]. The results globally support the model of 7B2–PC2 interaction illustrated in Figure 3. Pro7B2 attaches to proPC2 in the ER. This attachment is facilitated by the relatively alkaline conditions of this compartment. The inactive complex is transported to the TGN where pro7B2 is cleaved into an N-terminal protein and a C-terminal peptide. ProPC2 then gets autocatalytically cleaved after the prodomain as the complex is transported into secretory granules. In the acidic environment of these organelles, the prodomain and the 7B2 fragments dissociate from the enzyme, which then becomes fully active. Depending on experimental cell systems and conditions, variations from this model have been observed and are discussed in the following sections.

Results from truncation, domain swapping and site-directed-mutagenesis experiments have implicated various 7B2 domains or residues in the binding and activation of proPC2. Major determinants of these effects are clustered within an internal segment extending from residues 86 to 131. They include the Pro^{90}-Pro-Asn-Pro-Cys-Pro^{95} conserved motif, the Cys^{94}-Cys^{104} disulphide bridge, the α helical sequence between residues 108 and 121, as well as the segment between residues 121 and 131, particularly Tyr^{131} [65,80,81,83,85]. The Arg^{151}-Arg-Lys-Arg^{155} primary processing site has also been implicated in the interaction [79], but is not indispensable, since the $7B2^{1–150}$ processed form has been shown to interact with PC2 [85]. Strangely, the $7B2^{1–90}$ sequence, whose similarity with chaperonin 60 spurred this line of investigation [87], has no PC2-binding activity [88].

Reduced or non-glycosylated proPC2 cannot attach to 7B2, suggesting that its proper folding is a prerequisite for this interaction [80]. The oxyanion hole Asp^{309} and Tyr^{194} residues appear to be critical for both the binding of the zymogen to pro7B2 and its activation [79,82,84,89]. Interestingly, Tyr^{194} is located in a polyproline region (D^{192}**PY**PXPRY^{199}; where single-letter amino-acid notation has been used), which, like the one found in 7B2 (P^{88}DPPNPCP^{95}), bears some similarity with the Src homology 3 (SH3) domains [core motif PXXPX(R)]. On the basis of this similarity, an analogy was drawn between the

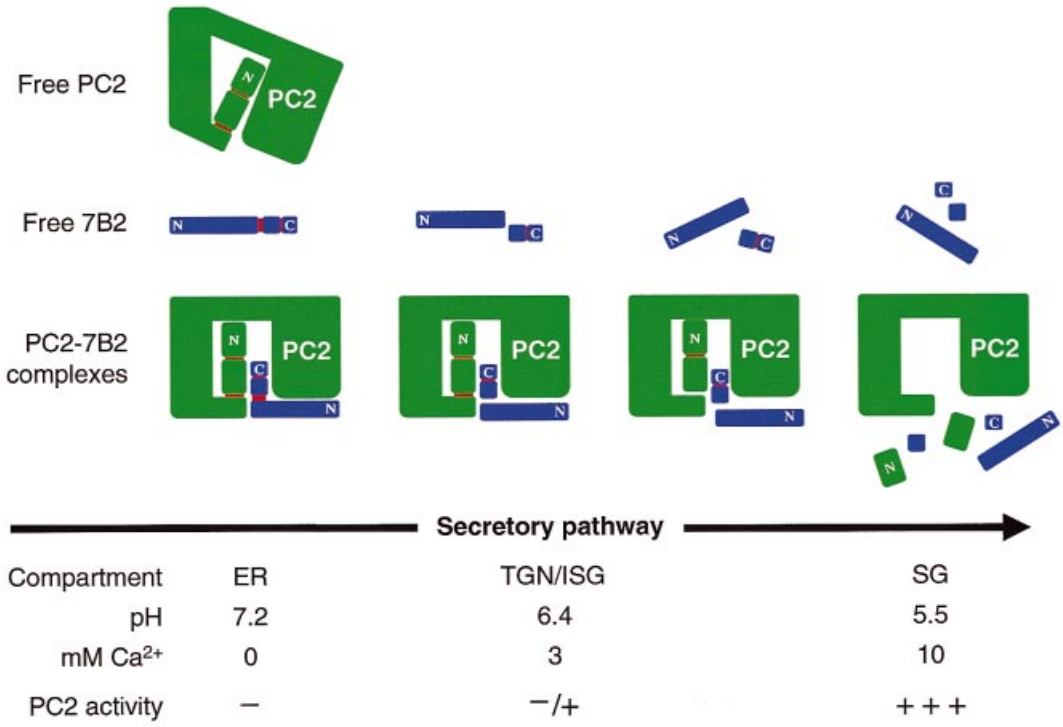

Figure 3 Schematic representation of 7B2 and PC2 transport in the secretory pathway

PC2 is presented as a folded green box with an extended bar at the N-terminus, and 7B2 is presented as a linear or bent blue box. Connecting red bars represent basic processing sites. According to the prevailing model of PC2 and 7B2 trafficking in the secretory pathway, free PC2 tends to misfold and to be retained in the ER, whereas free 7B2 or 7B2–PC2 complexes are more readily transported through the downstream compartments into secretory granules (SG). Affinity between the two molecules is influenced by, among other factors, the pH and the calcium concentration in the successive compartments of the secretory pathway. The pro7B2–proPC2 complex is formed in the alkaline and calcium-poor ER. It is enzymically inactive. Under the mildly acidic, calcium-richer conditions of the TGN and the immature secretory granules (ISG), pro7B2 is cleaved by furin, but its products remain attached to the PC2 zymogen, maintaining the latter in a maturation-conducive conformation. In the ISG, the zymogen self-activates by intramolecular cleavage after the prodomain, in a pH-driven calcium-independent manner. It is possible that the mature enzyme thus generated is transiently inhibited by the pro-region and the 7B2CT peptide. When the complex reaches the even more acidic and calcium-rich SG, these peptides are rapidly degraded by PC2 and CPE to non-inhibitory by-products, allowing the convertase to become fully active.

7B2–PC2 interaction with those mediated by SH3 domains [81,82]. The proregion and the P domain also contribute to the establishment and the stabilization of this interaction [84]. Sequence comparison between PC2 and PC1/3 has led to the identification within the catalytic domain of a unique seven residue (242–248) region of no homology. Substituting the PC1/3$^{242–248}$ region into PC2 reduced the ability of this enzyme to bind 7B2, without affecting its processing activity on pro-enkephalin or POMC [86]. Figure 4 illustrates the 7B2 and PC2 domains currently known to be relevant for interaction between the two molecules.

7B2 binding facilitates PC2 transport in the secretory pathway

Pulse–chase studies of newly synthesized proteins have shown that pro7B2 is transported out of the ER faster than proPC2, suggesting that the former needs not interact with the latter for its trafficking in the secretory pathway. Newly made proPC2, on the other hand, exits the ER much more slowly [5,88,90–92] and must acquire a proper conformation to do so. This conformational change is also required for the zymogen to bind passing pro7B2. Through this association, the conformation is presumably rendered more stable, allowing a faster migration from the ER to the Golgi [80]. Reduction of the bonded half-cystines of pro7B2 with DTT or their mutation to alanine abrogates its binding to proPC2 and causes retention of the zymogen in pre-Golgi compartments [65,80,81,83,85].

7B2 and PC2 proteins are both packaged into secretory granules. It is unclear whether proPC2 needs to be associated with 7B2 polypeptides to be sorted into these organelles. When 7B2 production was inhibited by antisense RNA in rat medullary thyroid carcinoma rMTC 6-23 cells, proPC2 could still convert into PC2 but was misdirected towards the constitutive secretory pathway [67], suggesting a need for 7B2 for the proper sorting of the enzyme. 7B2 binds PC2 with high affinity ($K_d \approx 7$ nM) [87,93]. Although not yet established, it may be a component of PC2 aggregates that are sorted into the secretory granules [94,95]. It has been shown to aggregate on its own in a calcium- and pH-dependent manner [96]. In part because of this property, it has been grouped with chromogranins and secretogranins into the so-called granin family of proteins, one of whose presumed functions is to facilitate the sorting of neuroendocrine proteins into secretory granules [97]. The strong affinity of 7B2 for PC2 is preserved when the whole molecule or its N-terminal fragment is fused to a heterologous protein. It has been ingeniously exploited by Rovère et al. [98] to deplete mouse corticotroph AtT-20 cells of PC2 by overexpression of a fusion 7B2 C-terminally tethered to the lysosomal cathepsin B. The depletion was due to PC2 binding to this chimaeric protein and to its misdirection towards the lysosomal degradation pathway.

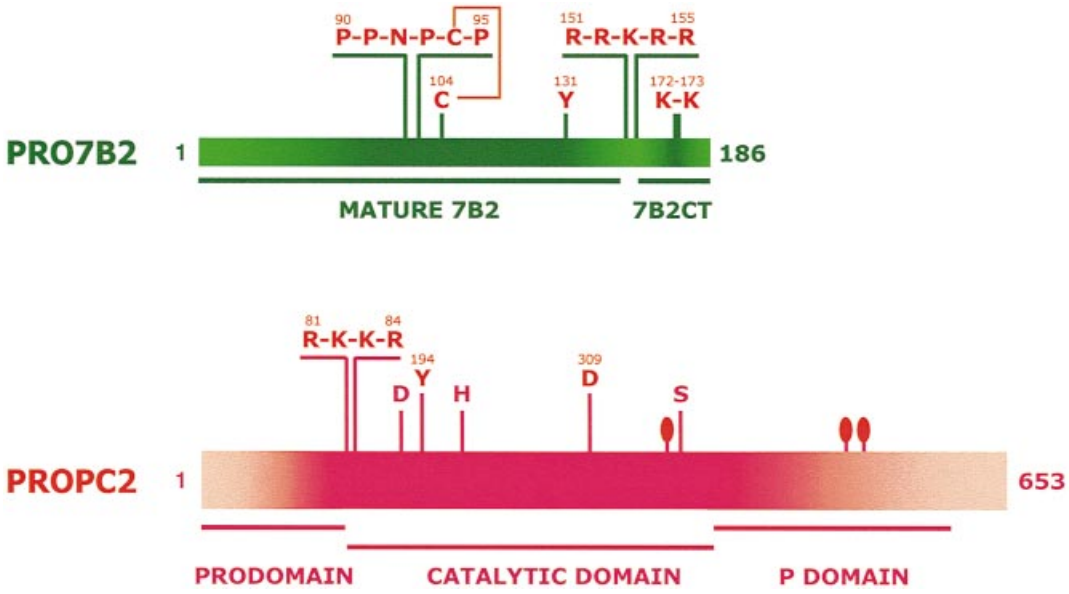

Figure 4 Graphical illustration of domains and residues involved in 7B2–PC2 interaction

Pro7B2 and proPC2 are represented linearly as coloured bars. The colour intensity reflects the relative importance of the various regions for effective interaction between the two molecules, as determined by deletional mutagenesis and site-directed mutagenesis. Residues that have been shown to be critical for the dynamics of this interaction (see the text) are indicated above the bars and are numbered. Most are located in the most intensely coloured regions of the bars. The major functional domains of the molecules are highlighted with lines below the bars and identified. For proPC2, glycosylation sites are indicated with small trees, and the aspartate, histidine and serine residues of the catalytic pocket are shown.

7B2 regulates PC2 activation

The interaction of proPC2 with 7B2 may be both facilitative and regulatory with regard to the maturation and activation of the enzyme. In some cells proPC2 is helped by, but does not absolutely require, 7B2 to proteolytically convert into PC2, as suggested in the above mentioned study with rMTC 6-23 cells expressing antisense 7B2 RNA [67]. In other cells, such as AtT-20/PC2 transfectants, transduced pro7B2 or $7B2^{1–150}$ clearly accelerates proPC2 maturation [88]. In the human neuroepithelioma SK-N-MCIXC cells, which express PC2 but not 7B2, the enzyme is secreted only as an inactive form of 71 kDa. Complementation of these cells with a 7B2 transgene led to the partial processing of endogenous proPC2 into the mature 68 kDa form [99], suggesting a need for 7B2 in proPC2 maturation.

Apparently, not all 7B2 molecular forms can promote this maturation. The precursor form, through its carboxy region (see below), may block, or at least interfere with, this maturation. Its prior processing might be required to allow proPC2 cleavage. Thus when supplemented to a cell-free translation lysate derived from *Xenopus* intermediate pituitary cells, mature 7B2, but not its unprocessed precursor, could stimulate PC2-mediated processing of POMC [100]. In the same vein, a processing-resistant mutant form of pro7B2, unlike the native form, was unable to promote the conversion of proPC2 into PC2 in mouse corticotroph AtT-20/PC2-transfectant cells [88]. This, however, may be due to the weaker affinity of the mutant pro7B2 for the zymogen [79] (leading to its retention in the ER), rather than to a direct maturation block. In a study with highly purified recombinant proPC2 obtained from CHO transfectant cells, neither pro7B2 nor its processing fragments were able to influence in any way the maturation of the zymogen, a process that appeared to be primarily pH-driven [101]. This study indicates that 7B2 may not facilitate proPC2 maturation in such a refined system, but does not contradict the likelihood of it doing so under the more complex conditions of the cellular secretory pathway.

C-terminal domain of 7B2 (7B2CT) peptide, a specific inhibitor of PC2

The 7B2CT peptide that follows the basic quintuplet has been shown to strongly inhibit PC2 activity *in vitro* at nanomolar levels [87,93]. The Lys^{172}-Lys^{173} pair is important for the binding and the inhibitory properties of the peptide [32,102,103]. These properties are conserved across species as they have been observed with *Lymneae* and *Caenorhabditis elegans* 7B2 [16,17]. However, there is no clear-cut experimental evidence showing that the 7B2CT peptide has any PC2-inhibitory activity *in vivo*. One study has suggested that pro7B2 can prevent proPC2 conversion into PC2 and that its processing and subsequent dissociation are prerequisites for the maturation of the zymogen [92]. Other studies have contradicted this conclusion by demonstrating that the processed 7B2 products remain associated with the zymogen, protecting it from denaturation and allowing its conversion into PC2 [80,104]. Moreover, when the 7B2CT peptide was tethered at the carboxy end of proenkephalin and transduced in mouse corticotroph AtT-20/PC2 transfectants or in rat insulinoma Rin cells, it failed to block PC2 processing of the prohormone [105]. The difficulty to detect any 7B2CT peptide inhibition of PC2 in cells may be due to the fact that, once activated in the increasingly acidic conditions of post-Golgi compartments [5,80,101,106], PC2 would rapidly degrade the peptide to non-inhibitory by-products that are further inactivated by CPE removal of C-terminal basic residues [32].

Clearly, the *in vitro* conditions that have permitted the identification of the inhibitory activity of the 7B2CT peptide can hardly be construed to replicate those existing in the secretory

compartments of cells. The dynamics of the interactions between PC2 and 7B2 in these compartments may be influenced by other parameters, including the composition, the relative concentration and the subcellular distribution of resident and transient components. It remains possible that the 7B2CT peptide, within the pro7B2 context, may indeed interfere with proPC2 conversion into PC2, but only in pre-TGN compartments. Once it is processed by furin in the TGN, its products remain bound to the zymogen but can no longer prevent the latter from being matured, either autocatalytically [107,108] or by related PCs, such as furin and PACE4 [79]. The association, in fact, would allow the zymogen to maintain a maturation-conducive conformation. Once matured, the enzyme may become susceptible to inhibition by both its own propeptide and by the 7B2CT peptide. The need for the latter peptide may be explained by the fact the PC2 propeptide, compared with that of PC1 and PC7 [109,110], is a relatively less potent inhibitor [108]. Whether and how it co-operates with the 7B2CT peptide to maintain the complex enzymically inactive is yet to be demonstrated and clarified. In mature granules the complex may be further loosened. PC2, in conjunction with CPE, would rapidly degrade the propeptide and the 7B2CT peptide into non-inhibitory by-products, thus becoming fully active (see Figure 3).

7B2 function in evolution

The regulatory effect of 7B2 on PC2 biosynthesis and activity extends across evolutionary distances, but its efficiency appears to be determined by intraspecific cellular characteristics. This was made most evident by studying the effect of invertebrate 7B2s on mammalian PC2. For example, full-length *Drosophila* 7B2 was shown to increase the secretion of PC2 activity into the culture medium when transduced into CHO cells overexpressing mouse PC2, as determined by the 7B2CT-inhibitable cleavage of a fluorogenic substrate. It was also able to induce this activity when added as a recombinant protein to Golgi-enriched membranes extracted from these cells [18]. This activity, however, was consistently lower than that induced by rat 7B2. In the same vein, the mature form of *C. elegans* 7B2 was also shown to promote PC2 activation secretion when expressed in these cells or supplemented *in vitro* to their Golgi membrane preparations, but again less efficiently than mature mouse 7B2 [17]. It is very likely that the biogenesis of 7B2 and PC2, as well as their effective interaction, is most adapted to the cellular environment of each species. Thus it was recently reported that *Drosophila* PC2 could not be matured, activated and secreted when transduced in mammalian cells, irrespective of the presence of *Drosophila* 7B2 or mouse 7B2. In contrast, it underwent the processing and secretion when transduced into S2 insect cells, but only in the presence of either insect 7B2 or rat 7B2 [18]. The interspecific inhibitory activity of the 7B2CT peptide on PC2 has also been examined by *in vitro* analysis of PC2-specific cleavage of a peptidyl fluorogenic substrate. Thus human CT7B2 was shown to inactivate *Drosophila* PC2, but 100-fold less efficiently than it did mouse PC2 [18]. Similarly, a *Lymneae* $7B2^{235–273}$ peptide was shown to inhibit mouse PC2, but 14-fold less potently that it did the orthologue PC2 activity contained in the neuroendocrine light green cell of the mollusc [16]. In the same vein, a full-length recombinant *C. elegans* 7B2 containing the CT region (but not its processed form lacking this region) was shown to inhibit purified mouse PC2 activity *in vitro*, but nearly 20-fold less efficiently than a full-length rat 7B2 [17].

Interestingly, *Lymneae* and *C. elegans* 7B2 sequences contain more than one region of partial similarity with the **VNPYL(Q/K)GKRLD-**NV**VAKKS**V**PH** (conserved residues are shown in bold and - indicates a gap) region encompassing the functionally crucial KK pair of mammalian 7B2CTs. Thus besides the **NPFLQG**EQM**D-**I**AAKK** (*Lymneae stagnalis* $7B2^{243–257}$) sequence found in the 7B2CT peptide, the *Lymneae* 7B2 also contains the upstream similar sequence **NPNLSSSRKRLLTLVAKKS-PH** (*L. stagnalis* $7B2^{209–229}$). Similarly, the *C. elegans* 7B2 contains the more remotely similar sequence **L**EENNM**H**A**NTIAKK** (*C. elegans* $7B2^{152–165}$), in addition to the C-terminal **VNPLYLQG**EP**L**R-SM**QKK** (*C. elegans* $7B2^{193–208}$). Functionally, a peptide encompassing the *L. stagnalis* $7B2^{209–229}$ region was shown to inhibit *Lymneae* PC2 as well as mouse PC2, but less efficiently than the more C-terminal peptide *L. stagnalis* $7B2^{235–273}$. Collectively, these observations suggest evolutionary conservation of the regulatory function of 7B2 on PC2 biosynthesis and activation, as well as adaptive co-evolution of the two genes during speciation. This view is supported by the striking similarity between the PC2 and 7B2 phylogenic trees derived from alignments of their respective sequences among human, pig, rat, *Xenopus*, *Drosophila* and *C. elegans* (Figure 5). These trees also suggest that 7B2 may have diverged more than PC2 after phylogenic separation between vertebrates and invertebrates. This divergence may reflect structural changes needed for the PC2-regulation function or acquisition of new functions by gain or loss of functional domains. Both hypotheses can be supported by the size and number of gaps needed to align vertebrate versus invertebrate 7B2 sequences (see Figure 1). Concerning the presence of multiple PC2-inhibitory domains in *C. elegans* 7B2 and *L. stagnalis* 7B2 sequences, this may have occurred through intergenetic exon shuffling or by sequence duplication during insertional recombination events.

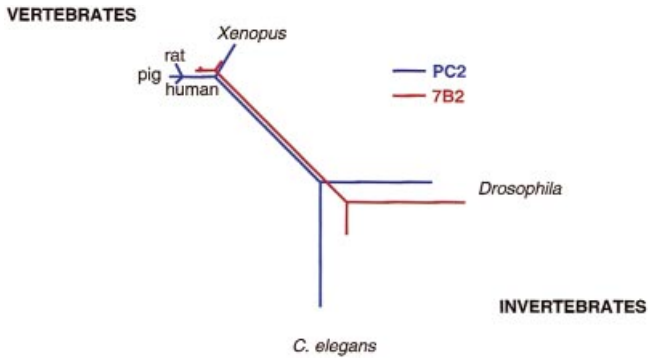

Figure 5 Co-evolution of pro7B2 and proPC2

To examine the evolution of pro7B2 and proPC2, phylum-representative species for which the complete protein sequences of both molecules have been elucidated were considered. These species include human, pig, rat, *Xenopus*, *Drosophila* and *C. elegans*. For each molecule, the sequences of the various species were aligned using the multiple alignment program CLUSTALW (http://evolution.genetics.washington.edu/phylip/software.etc1.html#ClustalW). The results were analysed using the neighbour-joining phylogeny program TREE (http://igs-server.cnrs-mrs.fr/anrs/phylogenetics) to derive the phylodendrons shown. There are general branching similarities between the two trees, as would be expected of functionally linked proteins. However, differences in the length of the branches can also be observed. They probably indicate that, along branches, each protein evolved at a distinct rate. Note how, in mammals, 7B2 sequences can hardly be distanced, as predictable from their homology (see Figure 1), whereas PC2 sequences can. In contrast, between vertebrates and invertebrates, the differentiation of 7B2 sequences is greater than that of PC2 sequences. Visual examination of the aligned sequences (see Figure 1 for 7B2) clearly shows that invertebrate 7B2 sequences have incorporated many more additional sequences during evolution than PC2 sequences. The tree patterns thus suggest both phylogenic links and differentiation in the functions of the two proteins.

Co-regulation of 7B2 and PC2

The functional link between PC2 and 7B2 also implies that expression of their genes could be co-ordinately regulated. Except for CREs, specificity protein 1 ('SP1'), AP1 and AP2-binding sites, the proximal promoter sequence of these genes share no other obvious regulatory elements [27,111]. Although there are many studies in which the regulation of either PC2 or 7B2 has been individually examined [27,68,112–117], there are very few that have concomitantly analysed the regulation of both proteins in a single organ, tissue or cell line. A co-ordinated increase in the intracellular levels of immunoreactive 7B2 and PC2 has been observed in rMTC 6-23 cells treated with dexamethasone for 1–2 days [67]. In another study [99], the adrenal levels of mRNAs for both proteins were shown to significantly decrease 6 h after insulin-induced hypoglycaemic shock; however, the level of 7B2 mRNA remained reduced 72 h later, whereas that of PC2 mRNA significantly increased 24 h later and remained high for 72 h. This discordance was associated with reduced conversion of proPC2 into PC2, suggesting that down-regulation of 7B2 may be a cellular mechanism of limiting the amount of PC2 activity. Petit-Turcotte and Paquin [118] examined the levels of immunoreactive PC2 and 7B2 in differentiated P19 neurons treated with signal transduction modulators acting through the thyroid hormone receptor (3,3′,5-tri-iodothyronine), the glucocorticoid receptor (dexamethasone), protein kinase C (PMA) and protein kinase A [dibutyryl cAMP or 3-isobutyl-1-methyl-xanthine (IBMX)]. Both 7B2 and PC2 levels slightly (less than 2-fold), but significantly, augmented after treatment with PMA and dibutyryl-cAMP. The increase was generally greater for PC2 that for 7B2. 3,3′,5-Tri-iodothyronine and IBMX increased the levels of PC2 but had no apparent effect on that of 7B2. Although dexamethasone alone had no effect, it attenuated the effect of PMA and accentuated that of IBMX [118]. These results suggest that, although co-ordinately regulated, 7B2 and PC2 expression may be differentially sensitive to regulatory agents.

Other biological roles of 7B2

Helping in the trafficking of PC2 and controlling the temporal activation of PC2 may not be the only cellular function of 7B2. Indeed, *in situ* hybridization studies on rat brain have shown that whereas all cells containing PC2 transcripts also contained 7B2 transcripts, there were many cells containing the latter without the former [99]. In a study of *X. laevis* ontogeny, 7B2 mRNA was found in unfertilized eggs, as well as in the earliest developmental stages, whereas PC2 mRNA became detectable only after embryonic neurogenesis [119].

Expression studies in yeast have raised the possibility that 7B2 may effectively interact with other molecules. When the human pro7B2 was expressed in yeast (*Saccharomyces cerevisiae*) containing the endogenous proprotein convertase Kex2 ($Kex2^+$ yeast), it remained unprocessed and was retained inside the cells, whereas a truncated 7B2 lacking the last 48 C-terminal residues was secreted. In pro7B2-expressing cells, Kex2 cleavage of a co-transduced proαfactor–IGF-1 fusion protein was inhibited, suggesting Kex2 inactivation by the C-terminal domain of 7B2. Interestingly, in Kex2-deficient ($Kex2^-$) yeast, pro7B2 was processed by an alternative protease activity that is induced by the mutation. Yap3, an aspartyl protease that also cuts after pairs of basic residues, is probably this alternative convertase since its coexpression with pro7B2 in $Kex2^+$ or $Kex2^-$ yeast promotes the processing and secretion of 7B2 products [120]. A coexpression study was also conducted to determine whether 7B2 can exert a chaperone-like effect on the biogenesis and secretion of IGF-1 which, when expressed in yeast, is secreted in multimeric form. 7B2 did not prevent the multimerization, but formed disulphide-linked complexes with IGF-1. However, when recombinant mature 7B2 was supplemented to the culture medium of recombinant yeast expressing IGF-1 alone, it promoted the formation of IGF-1 monomers in an ATP-dependent manner [121]. In a search for interacting proteins using a yeast two hybrid system, 7B2 was found to possess a potent intrinsic transcription-activating property, and to interact with the tumour suppressor protein p53 [121,122]. Since to date no 7B2-like molecule has been reported in yeast, these intriguing observations need to be confirmed and extended using cells that normally express 7B2.

The likelihood of other cellular roles for 7B2 has been made most evident by the remarkable phenotype of the 7B2 knockout mice generated by Westphal et al. [123]. Like in their PC2-knockout counterparts, PC2 activity is lacking in these mice and processing of pancreatic islet prohormones is severely impaired. However, unlike PC2 null mutants, which are viable, 7B2 null mutants die within weeks after birth from severe Cushing's disease due to excessive secretion of corticotropin by the intermediate lobe of the pituitary. It is unclear which of the various 7B2 peptides are involved in secretion regulation or whether they act in intracrine, autocrine or paracrine fashion. One of them, the C-terminal $7B2^{174–186}$ peptide, has been shown to induce membrane depolarization of VP and oxytocin of supraoptic nucleus neurons when applied to hypothalamic explants [124]. These observations need to be confirmed with other neuroendocrine systems.

7B2 AS A NEUROENDOCRINE MARKER

7B2 is detectable in human plasma. Its mean plasma level in healthy adults ranges from 40–140 pmol/litre [9,125–136]. It is remarkably high (as high as 1 nmol/litre) in early childhood, gradually decreases to adult levels by 20 years of age and slowly rises with aging [126,128]. It is elevated in pregnancy, from the second to the fourth trimester, but sharply declines soon after delivery, and returns to normal by 4–6 weeks post-partum [129]. The source of this supplemental circulating 7B2 in pregnancy is unclear. It is apparently not the fetus as suggested by the absence of a gradient of 7B2 concentrations in feto-maternal circulation. Indeed, although 7B2 is particularly elevated in fetal pancreas [137] and in cord blood [129,132,138], it is found in comparable amounts in the umbilical artery and the umbilical vein, in lower amounts in the maternal vein and in negligible amounts in the placenta [129,138]. The remarkable increase in plasma 7B2 in patients suffering from chronic kidney failure and liver cirrhosis [126,127,132], suggests that these organs are involved in its clearance.

The variations of circulating 7B2 probably reflect the biosynthetic and secretory states of a variety of neuroendocrine cells. However, the 7B2 reserve seems to be greater in some neuroendocrine cells than in others, as suggested by treatments of healthy individuals with cell-specific hypophysial hormone-releasing hormones. Thus a significant increase in plasma 7B2 was observed in such individuals following treatment with LHRH, but not following treatment with corticotropin-releasing hormone, thyrotropin-releasing hormone or GH-releasing hormone [128,131,136], supporting the preferential storage with pituitary gonadotropins. A correlation between plasma 7B2 and plasma gonadotropins was also reported in menopause and patients suffering from Klinefelter's syndrome, as well as in patients with various human chorionic gonadotropin-secreting tumours of the gastrointestinal tract [134]. In the same vein, 7B2 was shown to be actively secreted by functionless pituitary

Table 3 Conditions associated with elevated circulating levels of 7B2

Condition	References
Infancy (< 2 years)	[128]
Old age	[126,132]
Pregnancy (second and third trimesters)	[129,132]
Menopause	[134]
Klinefelter's syndrome	[134]
Diabetes + oral glucose	[132]
Chronic renal failure	[126,127,132]
Liver cirrhosis	[126,132]
Pituitary tumours	
Gonadotropinoma	[136]
Somatotroma (with acromegaly)	[131]
Medullary carcinoma of the thyroid	[130,141]
Gastrointestinal tract tumours	[125,134]
Pheochromocytoma	[125,130,141]
Small-cell lung carcinoma	[133]

adenoma, which are often associated with gonadotropin production [139]. High levels of circulating 7B2 have been detected in patients with other specific tumours of neuroendocrine origin. Thus higher than normal plasma 7B2 levels have been observed in acromegalic patients affected by a GH-producing microadenoma. These levels were further increased when the patients were intravenously injected with GH-releasing hormone or thyrotropin-releasing hormone [131], but decreased following treatment with the somatostatin analogue SMS 201-995 [140]. Likewise, patients with medullary carcinoma of the thyroid or pheochromocytoma showed elevated plasma 7B2 levels [130]. In contrast, these levels were within normal range in patients affected by Cushing's disease, prolactinoma or medullary carcinoma of the thyroid even after cell-specific exocytotic treatment [131,141]. Table 3 summarizes conditions known to be associated with elevated levels of circulating 7B2.

No study of circulating immunoreactive PC2 under normal and disease conditions has been reported to date to allow quantitative correlation between the enzyme and 7B2. Even if that were possible, it would still be challenging to link the two molecules to correlative variations of PC2 enzymic activity in particular organs or cells. At best, an indirect correlation could be inferred from corresponding changes in the circulating levels of bioactive peptides derived from established PC2 substrates.

Other biological fluids have been studied for their content of 7B2. The normal concentration of 7B2 in cerebrospinal fluid is 10–100-fold greater than that found in the plasma (0.7–2 nmol/litre) [142–144]. It decreases slightly with age [142], but is not significantly altered in cerebrovascular diseases [143,144]. It is also elevated in pleural effusion, with a mean concentration of approx. 600 pmol/litre, but does not vary significantly in benign or malignant pulmonary pathologies [145].

Immunological analysis of pathological tissues has revealed more extensive association of 7B2 with neuroendocrine tumours than titration of its circulating levels. 7B2 has been detected in nearly half of pediatric peripheral neuroectodermal tumours of the bone [146], in a majority of benign and approx. half of malignant pancreatic tumours, mostly in insulin-producing ones [125,137,147,148]. Interestingly, insulinomas containing only proinsulin and not its active mature form were found also to lack 7B2 [147], suggesting a possible link between the absence of the latter protein and the lack of PC2 activity. 7B2 was also present in a small fraction of medullary carcinoma of the thyroid [10,125,148], and among pituitary tumours, in gonadotropinoma and corticotroma [47,136,148], in benign and malignant phaeochromocytoma [125,148,149], in bronchial carcinoids and small-cell lung carcinoma [125,150,151].

Like 7B2, PC2 has been found to be a good marker of neuroendocrine tumours. It has been detected in pituitary adenomas [152,153], medullary thyroid carcinoma [154], pheochromocytomas [155,156], small-cell lung carcinomas [157,158], pancreatic islet tumours [159–161], and various carcinoids [161–163].

By examining neuroendocrine tumour samples, it has been possible to correlate expression of PC2 with the presence of peptides that this proteinase is known to generate from specific precursor proteins. Thus, in a study of POMC-expressing non-pituitary tumours, corticotropin-like intermediate lobe peptide ('CLIP'), a product of corticotropin cleavage by PC2, was found only in those tumours that express this convertase [148]. It can be assumed that these tumours also contained 7B2. A similar correlation involving both 7B2 and PC2 was drawn from the analysis of post-mortem hypothalamus samples from patients affected by the Prader–Willi syndrome (PWS), a neuroendocrine disorder associated with mutations on the paternal chromosome 15q11-q13 [164]. Because of the mapping of the 7B2 gene (*SGNE1*) in this region, it was considered to be a candidate gene for PWS [26]. In a semi-quantitative Southern-blot analysis of genomic DNA from a limited number of PWS patients, we observed no obvious anomaly of the 7B2 structural gene (M. G. Butler and M. Mbikay, unpublished work). However, in a post-mortem immunohistochemical study of supraoptic nuclei and paraventricular nuclei of five such patients, Gabreëls et al. [165] detected 7B2 in only three of the patients. The two patients lacking 7B2 in their hypothalamic nuclei also lacked PC2 and the processed form of VP [166], supporting the functional link between 7B2 and PC2 and the role of this enzyme in VP processing.

CONCLUDING REMARKS

The two-decade-long story of 7B2 has been marked by serendipitous turning points, which have made the importance of this molecule increasingly evident. It is still a matter of semantic debate whether 7B2 is an authentic molecular chaperone [78,80]. Its binding to proPC2, its stabilization of the zymogen in a transport-ready conformation, and its protective effect on PC2 against thermal- and pH-induced denaturation broadly meet some of the chaperone criteria [167]. However, its apparent lack of effect on proPC2 aggregation and folding puts it apart from classical chaperones, such as immunoglobulin heavy-chain binding protein ('BiP')/glucose-regulated protein of 78 kDa [168]. Like bacterial subtilisin [169], and mammalian furin [170], the folding of the PC2 zymogen primarily depends on its prodomain [107,108]. Pro7B2 binding may catalyse this folding towards a conformation that allows a faster exit from the ER. The co-operation between the prodomain and 7B2 may further extend to transient inhibition of PC2 activity, although this aspect remains to be convincingly demonstrated *in vivo*.

The PC2–7B2 model represents a novel neuroendocrine paradigm whereby endoproteolytic maturation of prohormones and proneuropeptides in the secretory pathway is spatially and temporally regulated by the dynamics of specific interactions between the PCs and resident binding proteins. The recent discovery of the proSAAS (where single-letter amino-acid notation has been used) molecule, which acts as a specific and potent inhibitor of PC1/3 [171–173] represents the first corroboration of this paradigm.

Involvement of 7B2 in the regulation of secretion is strongly suggested by the Cushing's disease of 7B2 knockout mice.

Whether, within the neuroendocrine system, this is a generalized or a cell-specific function, remains to be explored. Much is known about 7B2 biosynthesis and secretion, and little about the regulation of its expression at the levels of transcription and translation. Studies on the response of its mRNA and gene to physiological changes are likely to shed additional light on its other cellular functions. Finally, in view of the biological relevance of 7B2, it may be warranted to investigate whether structural or regulatory defects affecting its gene could be responsible for some human pathologies associated with abnormal processing and secretion of hormones and neuropeptides.

This work was supported by grants from the Canadian Institutes of Health Research and by the Canadian Diabetes Association. We are grateful to Dr Mieczyslaw Marcinkiewicz, Dr Claude Lazure and Dr Gilles Croissandeau for their critical review of this manuscript, and to Ms JoAnn Matheson for the search of the literature.

REFERENCES

1 Seidah, N. G. and Chrétien, M. (1997) Eukaryotic protein processing: endoproteolysis of precursor proteins. Curr. Opin. Biotechnol. **8**, 602–607
2 Seidah, N. G. and Chrétien, M. (1999) Proprotein and prohormone convertases: a family of subtilases generating diverse bioactive polypeptides. Brain Res. **848**, 45–62
3 Zhou, A., Webb, G., Zhu, X. and Steiner, D. F. (1999) Proteolytic processing in the secretory pathway. J. Biol. Chem. **274**, 20745–20748
4 Benjannet, S., Rondeau, N., Day, R., Chrétien, M. and Seidah, N. G. (1991) PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. Proc. Natl. Acad. Sci. U.S.A. **88**, 3564–3568
5 Benjannet, S., Rondeau, N., Paquet, L., Boudreault, A., Lazure, C., Chrétien, M. and Seidah, N. G. (1993) Comparative biosynthesis, covalent post-translational modifications and efficiency of prosegment cleavage of the prohormone convertases PC1 and PC2: glycosylation, sulphation and identification of the intracellular site of prosegment cleavage of PC1 and PC2. Biochem. J. **294**, 735–743
6 Hsi, K. L., Seidah, N. G., De Serres, G. and Chrétien, M. (1982) Isolation and NH_2-terminal sequence of a novel porcine anterior pituitary polypeptide. Homology to proinsulin, secretin and Rous sarcoma virus transforming protein TVFV60. FEBS Lett. **147**, 261–266
7 Seidah, N. G., Hsi, K. L., De Serres, G., Rochemont, J., Hamelin, J., Antakly, T., Cantin, M. and Chrétien, M. (1983) Isolation and NH_2-terminal sequence of a highly conserved human and porcine pituitary protein belonging to a new superfamily. Immunocytochemical localization in pars distalis and pars nervosa of the pituitary and in the supraoptic nucleus of the hypothalamus. Arch. Biochem. Biophys. **225**, 525–534
8 Iguchi, H., Chan, J. S., Seidah, N. G. and Chrétien, M. (1984) Tissue distribution and molecular forms of a novel pituitary protein in the rat. Neuroendocrinology **39**, 453–458
9 Iguchi, H., Chan, J. S., Seidah, N. G. and Chrétien, M. (1987) Evidence for a novel pituitary protein (7B2) in human brain, cerebrospinal fluid and plasma: brain concentrations in controls and patients with Alzheimer's disease. Peptides (N. Y.) **8**, 593–598
10 Marcinkiewicz, M., Benjannet, S., Falgueyret, J. P., Seidah, N. G., Schürch, W., Verdy, M., Cantin, M. and Chrétien, M. (1988) Identification and localization of 7B2 protein in human, porcine, and rat thyroid gland and in human medullary carcinoma. Endocrinology **123**, 866–873
11 Martens, G. J. (1988) Cloning and sequence analysis of human pituitary cDNA encoding the novel polypeptide 7B2. FEBS Lett. **234**, 160–164
12 Mbikay, M., Grant, S. G., Sirois, F., Tadros, H., Skowronski, J., Lazure, C., Seidah, N. G., Hanahan, D. and Chrétien, M. (1989) cDNA sequence of neuroendocrine protein 7B2 expressed in beta cell tumors of transgenic mice. Int. J. Pept. Protein Res. **33**, 39–45
13 Martens, G. J., Bussemakers, M. J., Ayoubi, T. A. and Jenks, B. G. (1989) The novel pituitary polypeptide 7B2 is a highly-conserved protein coexpressed with proopiomelanocortin. Eur. J. Biochem. **181**, 75–79
14 Waldbieser, G. C., Aimi, J. and Dixon, J. E. (1991) Cloning and characterization of the rat complementary deoxyribonucleic acid and gene encoding the neuroendocrine peptide 7B2. Endocrinology **128**, 3228–3236
15 Brayton, K. A., Aimi, J., Qiu, H., Yazdanparast, R., Ghatei, M. A., Polak, J. M., Bloom, S. R. and Dixon, J. E. (1988) Cloning, characterization, and sequence of a porcine cDNA encoding a secreted neuronal and endocrine protein. DNA **7**, 713–719
16 Spijker, S., Smit, A. B., Martens, G. J. and Geraerts, W. P. (1997) Identification of a molluscan homologue of the neuroendocrine polypeptide 7B2. J. Biol. Chem. **272**, 4116–4120
17 Lindberg, I., Tu, B., Muller, L. and Dickerson, I. M. (1998) Cloning and functional analysis of *C. elegans* 7B2. DNA Cell Biol. **17**, 727–734
18 Hwang, J. R., Siekhaus, D. E., Fuller, R. S., Taghert, P. H. and Lindberg, I. (2000) Interaction of Drosophila melanogaster prohormone convertase 2 and 7B2. Insect cell-specific processing and secretion. J. Biol. Chem. **275**, 17886–17893
19 Paquet, L., Lazure, C., Seidah, N. G., Chrétien, M. and Mbikay, M. (1991) The production by alternate splicing of two mRNAs differing by one codon could be an intrinsic property of neuroendocrine protein 7B2 gene expression in man. Biochem. Biophys. Res. Commun. **174**, 156–162
20 Lazure, C., Benjannet, S., Seidah, N. G. and Chrétien, M. (1991) Processed forms of neuroendocrine proteins 7B2 and secretogranin II are found in porcine pituitary extracts. Int. J. Pept. Protein Res. **38**, 392–400
21 Danoff, A. and Shields, D. (1988) Differential translation of two distinct preprosomatostatin messenger RNAs. J. Biol. Chem. **263**, 16461–16466
22 De Moor, C. H., Jansen, M., Sussenbach, J. S. and Van den Brande, J. L. (1994) Differential polysomal localization of human insulin-like-growth-factor-2 mRNAs in cell lines and foetal liver. Eur. J. Biochem. **222**, 1017–1024
23 Mathews, D. H., Sabina, J., Zuker, M. and Turner, D. H. (1999) Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. J. Mol. Biol. **288**, 911–940
24 Jacobson, A. and Peltz, S. W. (1996) Interrelationships of the pathways of mRNA decay and translation in eukaryotic cells. Annu. Rev. Biochem. **65**, 693–739
25 Ayala, S., Colomer, D., Gelpi, J. L. and Corrons, J. L. (1998) α-Thalassaemia due to a single codon deletion in the α1-globin gene. Computational structural analysis of the new α-chain variant. Hum. Mutat. **11**, 412
26 Mattei, M. G., Mbikay, M., Sylla, B. S., Lenoir, G., Mattei, J. F., Seidah, N. G. and Chrétien, M. (1990) Assignment of the gene for neuroendocrine protein 7B2 (SGNE1 locus) to mouse chromosome region 2[E3-F3] and to human chromosome region 15q11-q15. Genomics **6**, 436–440
27 Braks, J. A., Broers, C. A., Danger, J. M. and Martens, G. J. (1996) Structural organization of the gene encoding the neuroendocrine chaperone 7B2. Eur. J. Biochem. **236**, 60–67
28 Roebroek, A. J., Dehaen, M. R., van Bokhoven, A., Martens, G. J., Marÿnen, P., van den Berghe, H. and van de Ven, W. J. (1989) Regional mapping of the human gene encoding the novel pituitary polypeptide 7B2 to chromosome 15q13-q14 by in situ hybridization. Cytogenet. Cell Genet. **50**, 158–160
29 Paquet, L., Rondeau, N., Seidah, N. G., Lazure, C., Chrétien, M. and Mbikay, M. (1991) Immunological identification and sequence characterization of a peptide derived from the processing of neuroendocrine protein 7B2. FEBS Lett. **294**, 23–26
30 van Duijnhoven, H. L., Ayoubi, T. A., Timmer, E. D., Braks, A. A., Roebroek, A. J., Martens, G. J. and van de Ven, W. J. (1989) Development of a monoclonal antibody against recombinant neuroendocrine 7B2 protein. FEBS Lett. **255**, 372–376
31 van Duijnhoven, H. L., Verschuren, M. C., Timmer, E. D., Vissers, P. M., Groeneveld, A., Ayoubi, T. A., van den Ouweland, A. M. and van de Ven, W. J. (1991) Application of recombinant DNA technology in epitope mapping and targeting. Development and characterization of a panel of monoclonal antibodies against the 7B2 neuroendocrine protein. J. Immunol. Methods **142**, 187–198
32 Zhu, X., Rouille, Y., Lamango, N. S., Steiner, D. F. and Lindberg, I. (1996) Internal cleavage of the inhibitory 7B2 carboxyl-terminal peptide by PC2: a potential mechanism for its inactivation. Proc. Natl. Acad. Sci. U.S.A. **93**, 4919–4924
33 Marcinkiewicz, M., Benjannet, S., Cantin, M., Seidah, N. G. and Chrétien, M. (1986) CNS distribution of a novel pituitary protein '7B2': localization in secretory and synaptic vesicles. Brain Res. **380**, 349–356
34 Iguchi, H., Chan, J. S., Dennis, M., Seidah, N. G. and Chrétien, M. (1985) Regional distribution of a novel pituitary protein (7B2) in the rat brain. Brain Res. **338**, 91–96
35 Marcinkiewicz, M., Benjannet, S., Seidah, N. G., Cantin, M. and Chrétien, M. (1985) Immunocytochemical localization of a novel pituitary protein (7B2) within the rat brain and hypophysis. J. Histochem. Cytochem. **33**, 1219–1226
36 Marcinkiewicz, M., Touraine, P. and Chrétien, M. (1994) Pan-neuronal mRNA expression of the secretory polypeptide 7B2. Neurosci. Lett. **177**, 91–94
37 Marcinkiewicz, M., Benjannet, S., Sikström, R. A., Cantin, M., Seidah, N. G. and Chrétien, M. (1985) Immunoreactivity of vasopressin and a novel pituitary protein '7B2' in Long-Evans and Brattleboro rat hypothalamus and hypophysis. Neurosci. Lett. **60**, 7–12
38 Ayoubi, T. A., van Duijnhoven, H. L., Coenen, A. J., Jenks, B. G., Roubos, E. W. and Martens, G. J. (1991) Coordinated expression of 7B2 and αMSH in the melanotrope cells of Xenopus laevis. An immunocytochemical and in situ hybridization study. Cell Tissue Res. **264**, 329–334
39 Suzuki, H., Christofides, N. D., Anand, P., Chrétien, M., Seidah, N. G., Polak, J. M. and Bloom, S. R. (1985) Regional distribution of a novel pituitary protein (7B2) in the rat spinal cord: effect of neonatal capsaicin treatment and thoracic cord transection. Neurosci. Lett. **55**, 151–156

40 Marcinkiewicz, M., Fischer-Colbrie, R., Falgueyret, J. P., Benjannet, S., Seidah, N. G., Lazure, C., Winkler, H. and Chrétien, M. (1988) Two-dimensional immunoblotting analysis and immunocytochemical localization of the secretory polypeptide 7B2 in adrenal medulla. Neurosci. Lett. **95**, 81–87

41 Suzuki, H., Christofides, N. D., Ghiglione, M., Ferri, G. L., Chrétien, M., Seidah, N. G., Polak, J. M. and Bloom, S. R. (1988) Distribution of a novel pituitary protein (7B2) in mammalian gastrointestinal tract and pancreas. Dig. Dis. Sci. **33**, 718–723

42 Suzuki, H., Christofides, N. D., Adrian, T. E., Chrétien, M., Seidah, N. G., Polak, J. M. and Bloom, S. R. (1985) Ontogeny of a novel pituitary protein (7B2) in the human fetal intestine. Regul. Pept. **12**, 289–296

43 Falgueyret, J. P., Marcinkiewicz, M., Benjannet, S., Cantin, M., Seidah, N. G. and Chrétien, M. (1987) Immunocytochemical localization of a novel pituitary polypeptide 7B2 in the gastro-intestinal tract of the rat. Cell Tissue Res. **249**, 707–709

44 García-Caballero, A., Gallego, R., García-Caballero, T., Fraga, M., Blanco, M., Fernández-Redondo, V. and Beiras, A. (1997) Cellular and subcellular distribution of 7B2 in porcine Merkel cells. Anat. Rec. **248**, 159–163

45 Gabreëls, B. A., Verwer, R. W., Sonnemans, M. A., Sluiter, A. A., Ang, C. W. and van Leeuwen, F. W. (1997) Lack of translation of normal 7B2 mRNA levels in hypothalamic mutant vasopressin cells of the homozygous Brattleboro rat. Neurosci. Lett. **239**, 5–8

46 Gabreëls, B. A., Sonnemans, M. A., Seidah, N. G., Chrétien, M. and van Leeuwen, F. W. (1992) Dynamics of 7B2 and galanin expression in solitary magnocellular hypothalamic vasopressin neurons of the homozygous Brattleboro rat. Brain Res. **585**, 275–282

47 Steel, J. H., Van Noorden, S., Ballesta, J., Gibson, S. J., Ghatei, M. A., Burrin, J., Leonhardt, U., Domin, J., Bloom, S. R. and Polak, J. M. (1988) Localization of 7B2, neuromedin B, and neuromedin U in specific cell types of rat, mouse, and human pituitary, in rat hypothalamus, and in 30 human pituitary and extrapituitary tumors. Endocrinology **122**, 270–282

48 Marcinkiewicz, M., Benjannet, S., Seidah, N. G., Cantin, M. and Chrétien, M. (1987) The pituitary polypeptide 7B2 is associated with LH/FSH and TSH cells and is localized within secretory vesicles. Cell Tissue Res. **250**, 205–214

49 Marcinkiewicz, M., Touraine, P., Mbikay, M. and Chrétien, M. (1993) Expression of neuroendocrine secretory protein 7B2 mRNA in the mouse and rat pituitary gland. Neuroendocrinology **58**, 86–93

50 Seldeslagh, K. A. and Lauweryns, J. M. (1994) Immunohistochemical distribution of 7B2 and colocalization with calcitonin gene-related peptide in rat lung. Arch. Histol. Cytol. **57**, 359–367

51 Seldeslagh, K. A. and Lauweryns, J. M. (1994) 7B2 expression in intrapulmonary neuroepithelial bodies: immunocytochemical detection and colocalization with serotonin and calcitonin gene-related peptide. Regul. Pept. **53**, 223–236

52 Sánchez-Montesinos, I., Mérida-Velasco, J. A., Espín-Ferra, J. and Scopsi, L. (1996) Development of the sympathoadrenal system in the chick embryo: an immunocytochemical study with antibodies to pan-neuroendocrine markers, catecholamine-synthesizing enzymes, proprotein-processing enzymes, and neuropeptides. Anat. Rec. **245**, 94–101

53 Benjannet, S., Marcinkiewicz, M., Falgueyret, J. P., Johnson, D. E., Seidah, N. G. and Chrétien, M. (1988) Secretory protein 7B2 is associated with pancreatic hormones within normal islets and some experimentally induced tumors. Endocrinology **123**, 874–884

54 Ayoubi, T. A., van Duijnhoven, H. L., van de Ven, W. J., Jenks, B. G., Roubos, E. W. and Martens, G. J. (1990) The neuroendocrine polypeptide 7B2 is a precursor protein. J. Biol. Chem. **265**, 15644–15647

55 Paquet, L., Bergeron, F., Boudreault, A., Seidah, N. G., Chrétien, M., Mbikay, M. and Lazure, C. (1994) The neuroendocrine precursor 7B2 is a sulfated protein proteolytically processed by a ubiquitous furin-like convertase. J. Biol. Chem. **269**, 19279–19285

56 Sigafoos, J., Chestnut, W. G., Merrill, B. M., Taylor, L. C., Diliberto, E. J. J. and Viveros, O. H. (1993) Identification of a 7B2-derived tridecapeptide from bovine adrenal medulla chromaffin vesicles. Cell. Mol. Neurobiol. **13**, 271–278

57 Seidah, N. G., Chrétien, M. and Day, R. (1994) The family of subtilisin/kexin like pro-protein and pro-hormone convertases: divergent or shared functions. Biochimie **76**, 197–209

58 Zhong, M., Benjannet, S., Lazure, C., Munzer, S. and Seidah, N. G. (1996) Functional analysis of human PACE4-A and PACE4-C isoforms: identification of a new PACE4-CS isoform. FEBS Lett. **396**, 31–36

59 Benjannet, S., Savaria, D., Laslop, A., Munzer, J. S., Chrétien, M., Marcinkiewicz, M. and Seidah, N. G. (1997) α1-Antitrypsin Portland inhibits processing of precursors mediated by proprotein convertases primarily within the constitutive secretory pathway. J. Biol. Chem. **272**, 26210–26218

60 Deng, J. Y., Chan, J. S., Seidah, N. G. and Chrétien, M. (1986) Specific release of a novel pituitary polypeptide, 7B2, from rat anterior pituitary cells in vitro by luteinizing hormone-releasing hormone. Neuroendocrinology **44**, 373–377

61 Chan, J. S., Deng, J. Y., Brar, A. K., Seidah, N. G. and Chrétien, M. (1986) Inhibition of release of a novel pituitary polypeptide, 7B2, follicle-stimulating hormone, and luteinizing hormone from rat anterior pituitary cells in vitro by human β-inhibin. Can. J. Physiol. Pharmacol. **64**, 1259–1262

62 Iguchi, H., Natori, S., Nawata, H., Kato, K., Ibayashi, H., Chan, J. S., Seidah, N. G. and Chrétien, M. (1987) Presence of the novel pituitary protein 7B2 in bovine chromaffin granules: possible co-release of 7B2 and catecholamine as induced by nicotine. J. Neurochem. **49**, 1810–1814

63 Suzuki, H., Tischler, A. S., Christofides, N. D., Chrétien, M., Seidah, N. G., Polak, J. M. and Bloom, S. R. (1986) A novel pituitary protein (7B2)-like immunoreactivity is secreted by a rat phaeochromocytoma cell line (PC12). J. Endocrinol. **108**, 151–155

64 Iguchi, H., Okeda, T. and Takaki, R. (1991) Evidence for secretion of 7B2 by A- and B-cells of hamster pancreatic islets. Regul. Pept. **36**, 407–414

65 Van Horssen, A. M., Van Kuppeveld, F. J. and Martens, G. J. (1998) Manipulation of disulfide bonds differentially affects the intracellular transport, sorting, and processing of neuroendocrine secretory proteins. J. Neurochem. **71**, 402–409

66 Jeannotte, R., Paquin, J., Petit-Turcotte, C. and Day, R. (1997) Convertase PC2 and the neuroendocrine polypeptide 7B2 are co-induced and processed during neuronal differentiation of P19 embryonal carcinoma cells. DNA Cell Biol. **16**, 1175–1187

67 Barbero, P. and Kitabgi, P. (1999) Protein 7B2 is essential for the targeting and activation of PC2 into the regulated secretory pathway of rMTC 6-23 cells. Biochem. Biophys. Res. Commun. **257**, 473–479

68 Gherzi, R., Fehmann, H. C., Eissele, R. and Göke, B. (1994) Expression, intracellular localization, and gene transcription regulation of the secretory protein 7B2 in endocrine pancreatic cell lines and human insulinomas. Exp. Cell Res. **213**, 20–27

69 Tsuchiya, T., Suzuki, Y., Suzuki, H., Ohtake, R. and Shimoda, S. I. (1990) Changes in adrenal neuropeptides content [peptide 7B2, neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP)] induced by pharmacological and hormonal manipulations. J. Endocrinol. Invest. **13**, 381–389

70 van Leeuwen, F. W. (1992) Mutant vasopressin precursor producing cells of the homozygous Brattleboro rat as a model for co-expression of neuropeptides. Prog. Brain Res. **92**, 149–155

71 Sonenberg, N. (1993) Translation factors as effectors of cell growth and tumorigenesis. Curr. Opin. Cell Biol. **5**, 955–960

72 Kozak, M. (1991) Structural features in eukaryotic mRNAs that modulate the initiation of translation. J. Biol. Chem. **266**, 19867–19870

73 Kozak, M. (1992) Regulation of translation in eukaryotic systems. Annu. Rev. Cell Biol. **8**, 197–225

74 Kozak, M. (1994) Determinants of translational fidelity and efficiency in vertebrate mRNAs. Biochimie **76**, 815–821

75 Kozak, M. (1991) An analysis of vertebrate mRNA sequences: intimations of translational control. J. Cell Biol. **115**, 887–903

76 Guest, P. C., Arden, S. D., Bennett, D. L., Clark, A., Rutherford, N. G. and Hutton, J. C. (1992) The post-translational processing and intracellular sorting of PC2 in the islets of Langerhans. J. Biol. Chem. **267**, 22401–22406

77 Skelly, R. H., Schuppin, G. T., Ishihara, H., Oka, Y. and Rhodes, C. J. (1996) Glucose-regulated translational control of proinsulin biosynthesis with that of the proinsulin endopeptidases PC2 and PC3 in the insulin-producing MIN6 cell line. Diabetes **45**, 37–43

78 Braks, J. A. and Martens, G. J. (1994) 7B2 is a neuroendocrine chaperone that transiently interacts with prohormone convertase PC2 in the secretory pathway. Cell (Cambridge, Mass.) **78**, 263–273

79 Benjannet, S., Savaria, D., Chrétien, M. and Seidah, N. G. (1995) 7B2 is a specific intracellular binding protein of the prohormone convertase PC2. J. Neurochem. **64**, 2303–2311

80 Muller, L., Zhu, X. and Lindberg, I. (1997) Mechanism of the facilitation of PC2 maturation by 7B2: involvement in ProPC2 transport and activation but not folding. J. Cell Biol. **139**, 625–638

81 Zhu, X., Lamango, N. S. and Lindberg, I. (1996) Involvement of a polyproline helix-like structure in the interaction of 7B2 with prohormone convertase 2. J. Biol. Chem. **271**, 23582–23587

82 Benjannet, S., Mamarbachi, A. M., Hamelin, J., Savaria, D., Munzer, J. S., Chrétien, M. and Seidah, N. G. (1998) Residues unique to the pro-hormone convertase PC2 modulate its autoactivation, binding to 7B2 and enzymatic activity. FEBS Lett. **428**, 37–42

83 Van Horssen, A. M. and Martens, G. J. (1998) Mapping of the domain in the neuroendocrine protein 7B2 important for its helper function towards prohormone convertase PC2. Mol. Cell. Endocrinol. **137**, 7–12

84 Zhu, X., Muller, L., Mains, R. E. and Lindberg, I. (1998) Structural elements of PC2 required for interaction with its helper protein 7B2. J. Biol. Chem. **273**, 1158–1164

85 Muller, L., Zhu, P., Juliano, M. A., Juliano, L. and Lindberg, I. (1999) A 36-residue peptide contains all of the information required for 7B2-mediated activation of prohormone convertase 2. J. Biol. Chem. **274**, 21471–21477

86 Apletalina, E. V., Muller, L. and Lindberg, I. (2000) Mutations in the catalytic domain of prohormone convertase 2 result in decreased binding to 7B2 and loss of inhibition with 7B2 C-terminal peptide. J. Biol. Chem. **275**, 14667–14677

87 Martens, G. J., Braks, J. A., Eib, D. W., Zhou, Y. and Lindberg, I. (1994) The neuroendocrine polypeptide 7B2 is an endogenous inhibitor of prohormone convertase PC2. Proc. Natl. Acad. Sci. U.S.A. **91**, 5784–5787

88 Zhu, X. and Lindberg, I. (1995) 7B2 facilitates the maturation of proPC2 in neuroendocrine cells and is required for the expression of enzymatic activity. J. Cell Biol. **129**, 1641–1650

89 Benjannet, S., Lusson, J., Hamelin, J., Savaria, D., Chrétien, M. and Seidah, N. G. (1995) Structure-function studies on the biosynthesis and bioactivity of the precursor convertase PC2 and the formation of the PC2/7B2 complex. FEBS Lett. **362**, 151–155

90 Shen, F. S., Seidah, N. G. and Lindberg, I. (1993) Biosynthesis of the prohormone convertase PC2 in Chinese hamster ovary cells and in rat insulinoma cells. J. Biol. Chem. **268**, 24910–24915

91 Zhou, A. and Mains, R. E. (1994) Endoproteolytic processing of proopiomelanocortin and prohormone convertases 1 and 2 in neuroendocrine cells overexpressing prohormone convertases 1 or 2. J. Biol. Chem. **269**, 17440–17447

92 Braks, J. A., Van Horssen, A. M. and Martens, G. J. (1996) Dissociation of the complex between the neuroendocrine chaperone 7B2 and prohormone convertase PC2 is not associated with proPC2 maturation. Eur. J. Biochem. **238**, 505–510

93 Lindberg, I., van den Hurk, W. H., Bui, C. and Batie, C. J. (1995) Enzymatic characterization of immunopurified prohormone convertase 2: potent inhibition by a 7B2 peptide fragment. Biochemistry (Moscow) **34**, 5486–5493

94 Shennan, K. I., Taylor, N. A. and Docherty, K. (1994) Calcium- and pH-dependent aggregation and membrane association of the precursor of the prohormone convertase PC2. J. Biol. Chem. **269**, 18646–18650

95 Jan, G., Taylor, N. A., Scougall, K. T., Docherty, K. and Shennan, K. I. (1998) The propeptide of prohormone convertase PC2 acts as a transferable aggregation and membrane-association signal. Eur. J. Biochem. **257**, 41–46

96 Linard, C. G., Tadros, H., Sirois, F. and Mbikay, M. (1995) Calcium-induced aggregation of neuroendocrine protein 7B2 in vitro and its modulation by ATP. Mol. Cell. Biochem. **151**, 39–47

97 Ozawa, H. and Takata, K. (1995) The granin family – its role in sorting and secretory granule formation. Cell Struct. Funct. **20**, 415–420

98 Rovère, C., Mort, J. S., Chrétien, M. and Seidah, N. G. (2000) Cathepsin-B fusion proteins misroute secretory protein partners as the proprotein convertase PC2-7B2 complex toward the lysosomal degradative pathways. Biochem. Biophys. Res. Commun. **276**, 594–599

99 Seidel, B., Dong, W., Savaria, D., Zheng, M., Pintar, J. E. and Day, R. (1998) Neuroendocrine protein 7B2 is essential for proteolytic conversion and activation of proprotein convertase 2 in vivo. DNA Cell Biol. **17**, 1017–1029

100 Braks, J. A. and Martens, G. J. (1995) The neuroendocrine chaperone 7B2 can enhance in vitro POMC cleavage by prohormone convertase PC2. FEBS Lett. **371**, 154–158

101 Lamango, N. S., Apletalina, E., Liu, J. and Lindberg, I. (1999) The proteolytic maturation of prohormone convertase 2 (PC2) is a pH-driven process. Arch. Biochem. Biophys. **362**, 275–282

102 van Horssen, A. M., van den Hurk, W. H., Bailyes, E. M., Hutton, J. C., Martens, G. J. and Lindberg, I. (1995) Identification of the region within the neuroendocrine polypeptide 7B2 responsible for the inhibition of prohormone convertase PC2. J. Biol. Chem. **270**, 14292–14296

103 Apletalina, E. V., Juliano, M. A., Juliano, L. and Lindberg, I. (2000) Structure-function analysis of the 7B2 CT peptide. Biochem. Biophys. Res. Commun. **267**, 940–942

104 Lamango, N. S., Zhu, X. and Lindberg, I. (1996) Purification and enzymatic characterization of recombinant prohormone convertase 2: stabilization of activity by 21 kDa 7B2. Arch. Biochem. Biophys. **330**, 238–250

105 Fortenberry, Y., Liu, J. and Lindberg, I. (1999) The role of the 7B2 CT peptide in the inhibition of prohormone convertase 2 in endocrine cell lines. J. Neurochem. **73**, 994–1003

106 Shennan, K. I., Taylor, N. A., Jermany, J. L., Matthews, G. and 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. J. Biol. Chem. **270**, 1402–1407

107 Matthews, G., Shennan, K. I., Seal, A. J., Taylor, N. A., Colman, A. and Docherty, K. (1994) Autocatalytic maturation of the prohormone convertase PC2. J. Biol. Chem. **269**, 588–592

108 Muller, L., Cameron, A., Fortenberry, Y., Apletalina, E. V. and Lindberg, I. (2000) Processing and sorting of the prohormone convertase 2 propeptide. J. Biol. Chem. **275**, 39213–39222

109 Boudreault, A., Gauthier, D. and Lazure, C. (1998) Proprotein convertase PC1/3-related peptides are potent slow tight-binding inhibitors of murine PC1/3 and hfurin. J. Biol. Chem. **273**, 31574–31580

110 Zhong, M., Munzer, J. S., Basak, A., Benjannet, S., Mowla, S. J., Decroly, E., Chrétien, M. and Seidah, N. G. (1999) The prosegments of furin and PC7 as potent inhibitors of proprotein convertases. In vitro and ex vivo assessment of their efficacy and selectivity. J. Biol. Chem. **274**, 33913–33920

111 Ohagi, S., LaMendola, J., LeBeau, M. M., Espinosa, III, R., Takeda, J., Smeekens, S. P., Chan, S. J. and Steiner, D. F. (1992) Identification and analysis of the gene encoding human PC2, a prohormone convertase expressed in neuroendocrine tissues. Proc. Natl. Acad. Sci. U.S.A. **89**, 4977–4981

112 Birch, N. P., Tracer, H. L., Hakes, D. J. and Loh, Y. P. (1991) Coordinate regulation of mRNA levels of pro-opiomelanocortin and the candidate processing enzymes PC2 and PC3, but not furin, in rat pituitary intermediate lobe. Biochem. Biophys. Res. Commun. **179**, 1311–1319

113 Day, R., Schafer, M. K., Watson, S. J., Chrétien, M. and Seidah, N. G. (1992) Distribution and regulation of the prohormone convertases PC1 and PC2 in the rat pituitary. Mol. Endocrinol. **6**, 485–497

114 Oyarce, A. M., Hand, T. A., Mains, R. E. and Eipper, B. A. (1996) Dopaminergic regulation of secretory granule-associated proteins in rat intermediate pituitary. J. Neurochem. **67**, 229–241

115 Schuppin, G. T. and Rhodes, C. J. (1996) Specific co-ordinated regulation of PC3 and PC2 gene expression with that of preproinsulin in insulin-producing βTC3 cells. Biochem. J. **313**, 259–268

116 Nie, Y., Nakashima, M., Brubaker, P. L., Li, Q. L., Perfetti, R., Jansen, E., Zambre, Y., Pipeleers, D. and Friedman, T. C. (2000) Regulation of pancreatic PC1 and PC2 associated with increased glucagon-like peptide 1 in diabetic rats. J. Clin. Invest. **105**, 955–965

117 Li, Q. L., Jansen, E., Brent, G. A., Naqvi, S., Wilber, J. F. and Friedman, T. C. (2000) Interactions between the prohormone convertase 2 promoter and the thyroid hormone receptor. Endocrinology **141**, 3256–3266

118 Petit-Turcotte, C. and Paquin, J. (2000) Coordinate regulation of neuroendocrine convertase PC2 and peptide 7B2 in P19 neurons. Peptides (N.Y.) **21**, 365–372

119 Holling, T. M., van Herp, F., Durston, A. J. and Martens, G. J. (2000) Differential onset of expression of mRNAs encoding proopiomelanocortin, prohormone convertases 1 and 2, and granin family members during Xenopus laevis development. Brain Res. Mol. Brain Res. **75**, 70–75

120 Chaudhuri, B. and Stephan, C. (1995) A C-terminal domain, which prevents secretion of the neuroendocrine protein 7B2 in Saccharomyces cerevisiae, inhibits Kex2 yet is processed by the Yap3 protease. FEBS Lett. **364**, 91–97

121 Chaudhuri, B., Stephan, C., Huijbregts, R. P. and Martens, G. J. (1995) The neuroendocrine protein 7B2 acts as a molecular chaperone in the in vitro folding of human insulin-like growth factor-1 secreted from yeast. Biochem. Biophys. Res. Commun. **211**, 417–425

122 Chaudhuri, B., Huijbregts, R. P., Coen, J. J. and Fürst, P. (1995) The neuroendocrine protein 7B2 contains unusually potent transcriptional activating sequences. Biochem. Biophys. Res. Commun. **216**, 1–10

123 Westphal, C. H., Muller, L., Zhou, A., Zhu, X., Bonner-Weir, S., Schambelan, M., Steiner, D. F., Lindberg, I. and Leder, P. (1999) The neuroendocrine protein 7B2 is required for peptide hormone processing in vivo and provides a novel mechanism for pituitary Cushing's disease. Cell (Cambridge, Mass.) **96**, 689–700

124 Senatorov, V. V., Yang, C. R., Marcinkiewicz, M., Chrétien, M. and Renaud, L. P. (1993) Depolarizing action of secretory granule protein 7B2 on rat supraoptic neurosecretory neurons. J. Neuroendocrinol. **5**, 533–536

125 Suzuki, H., Ghatei, M. A., Williams, S. J., Uttenthal, L. O., Facer, P., Bishop, A. E., Polak, J. M. and Bloom, S. R. (1986) Production of pituitary protein 7B2 immunoreactivity by endocrine tumors and its possible diagnostic value. J. Clin. Endocrinol. Metab. **63**, 758–765

126 Natori, S., Iguchi, H., Nawata, H., Kato, K., Ibayashi, H. and Chrétian, M. (1987) Age-related change in plasma concentration of 7B2 (a novel pituitary polypeptide) in normal humans. Life Sci. **41**, 977–981

127 Iguchi, H., Natori, S., Ou, Y., Nawata, H., Kato, K., Ibayashi, H. and Bloom, S. R. (1988) Plasma levels of 7B2 (a novel pituitary polypeptide) and its molecular forms in plasma and urine in patients with chronic renal failure: possible degradation by the kidney. Regul. Pept. **21**, 263–270

128 Iguchi, H., Niida, Y., Natori, S., Takayanagi, N., Kohno, H., Kato, K. and Nawata, H. (1988) Remarkable changes in the plasma levels of pituitary protein 7B2 during childhood. Pediatr. Res. **24**, 194–196

129 Leonhardt, U. T., Stevenson, J. C., Ghatei, M. A., Lischka, A., Macdonald, D. W., Whitehead, M. I. and Bloom, S. R. (1988) Elevated 7B2 levels during normal human pregnancy. Am. J. Obstet. Gynecol. **158**, 1141–1144

130 Natori, S., Iguchi, H., Ohashi, M. and Nawata, H. (1988) Plasma 7B2 (a novel pituitary protein) immunoreactivity concentrations in patients with various endocrine disorders. Endocrinol. Jpn. **35**, 651–654

131 Natori, S., Iguchi, H., Nawata, H., Kato, K., Ibayashi, H., Nakagaki, H. and Chrétien, M. (1988) Evidence for the release of a novel pituitary polypeptide (7B2) from the growth hormone-producing pituitary adenoma of patients with acromegaly. J. Clin. Endocrinol. Metab. **66**, 430–437

132 Suzuki, H., Kobori, H., Ohtake, R., Hashigami, Y., Suzuki, Y., Shimoda, S. I. and Bloom, S. R. (1988) Circulating concentrations of immunoreactive peptide 7B2 in certain pathophysiological conditions, and response to oral glucose load. Clin. Chem. (Washington, D.C.) **34**, 410–413

133 Iguchi, H., Hara, N., Hayashi, I., Ohta, M., Bloom, S. R. and Chrétien, M. (1989) Elevation of a novel pituitary protein (7B2) in the plasma in small cell carcinoma of the lung. Eur. J. Cancer Clin. Oncol. **25**, 1225–1232

134 Leonhardt, U., Eriksson, B., Oberg, K., Wide, L., Ghatei, M. A. and Bloom, S. R. (1989) A new pituitary protein 7B2 is increased in patients with high α- or β-hCG. Acta Endocrinol. **120**, 289–294

135 Natori, S., Iguchi, H., Ohashi, M., Chrétien, M. and Nawata, H. (1989) LHRH increases plasma 7B2 concentration in normal human subjects. Endocrinol. Jpn. **36**, 367–371

136 Iguchi, H., Demura, R., Yasuda, D. and Wakasugi, H. (1992) Effect of LHRH on plasma 7B2 in patients with gonadotropin-producing pituitary adenomas. Horm. Metab. Res. **24**, 31–33

137 Suzuki, H., Christofides, N. D., Chrétien, M., Seidah, N. G., Polak, J. M. and Bloom, S. R. (1987) Developmental changes in immunoreactive content of novel pituitary protein 7B2 in human pancreas and its identification in pancreatic tumors. Diabetes **36**, 1276–1279

138 Iguchi, H., Natori, S., Nawata, H., Kato, K., Ibayashi, H., Chan, J. S., Seidah, N. G. and Chrétien, M. (1987) Elevation of plasma 7B2 (a novel pituitary protein) in cord blood at obstetrical delivery and the possible correlation with GH. Life Sci. **41**, 1921–1927

139 Venetikou, M. S., Ghatei, M. A., Burrin, J. M., Latif, S. and Bloom, S. R. (1988) 7B2, a new protein secreted by human functionless pituitary tumours, in vitro. Acta Endocrinol. **118**, 521–527

140 Natori, S., Iguchi, H., Ohashi, M., Nakao, R., Bloom, S. R. and Nawata, H. (1990) Effect of octapeptide somatostatin analogue (SMS 201–995) on plasma 7B2 (a neuroendocrine polypeptide) levels in patients with acromegaly. Clin. Endocrinol. **32**, 49–55

141 Ohashi, M., Natori, S., Fujio, N., Iguchi, H. and Nawata, H. (1990) Secretory protein 7B2. A novel tumor marker of medullary carcinoma of the thyroid. Horm. Metab. Res. **22**, 114–116

142 Natori, S., Iguchi, H., Kurose, S., Bloom, S. R. and Nawata, H. (1988) Age-related change in 7B2 (a novel pituitary polypeptide) concentrations in human cerebrospinal fluid. Regul. Pept. **22**, 371–376

143 Suzuki, H., Sato, S., Suzuki, Y., Ohtake, R., Hashigami, Y., Namba, J., Ishihara, N. and Shimoda, S. (1989) Pituitary protein 7B2-like immunoreactivity in cerebrospinal fluid: comparison with other neuropeptides. J. Lab. Clin. Med. **113**, 743–748

144 Suzuki, Y., Sato, S., Suzuki, H., Namba, J., Ohtake, R., Hashigami, Y., Suga, S., Ishihara, N. and Shimoda, S. (1989) Increased neuropeptide Y concentrations in cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage. Stroke **20**, 1680–1684

145 Iguchi, H., Hara, N., Miyazaki, K., Natori, S., Nawata, H., Ohta, M. and Bloom, S. R. (1988) Presence of high concentration of 7B2 in pleural effusion. Endocrinol. Jpn. **35**, 885–889

146 Collini, P., Sampietro, G., Luksch, R., Migliorini, L., Boracchi, P. and Scopsi, L. (1998) Differentiation in paediatric peripheral primitive neuroectodermal tumours of bone. A critical contribution to its assessment. Virchows Arch. **432**, 505–513

147 Azzoni, C., Yu, J. Y., Baggi, M. T., D'Adda, T., Timson, C., Polak, J. M. and Bordi, C. (1992) Studies on co-localization of 7B2 and pancreatic hormones in normal and tumoural islet cells. Virchows Arch. A: Pathol. Anat. Histopathol. **421**, 457–466

148 Vieau, D., Seidah, N. G., Mbikay, M., Chrétien, M. and Bertagna, X. (1994) Expression of the prohormone convertase PC2 correlates with the presence of corticotropin-like intermediate lobe peptide in human adrenocorticotropin-secreting tumors. J. Clin. Endocrinol. Metab. **79**, 1503–1506

149 Hacker, G. W., Bishop, A. E., Terenghi, G., Varndell, I. M., Aghahowa, J., Pollard, K., Thurner, J. and Polak, J. M. (1988) Multiple peptide production and presence of general neuroendocrine markers detected in 12 cases of human phaeochromocytoma and in mammalian adrenal glands. Virchows Arch. A: Pathol. Anat. Histopathol. **412**, 399–411

150 Roebroek, A. J., Martens, G. J., Duits, A. J., Schalken, J. A., van Bokhoven, A., Wagenaar, S. S. and van de Ven, W. J. (1989) Differential expression of the gene encoding the novel pituitary polypeptide 7B2 in human lung cancer cells. Cancer Res. **49**, 4154–4158

151 Vieau, D., Linard, C. G., Mbikay, M., Lenne, F., Chrétien, M., Luton, J. P. and Bertagna, X. (1992) Expression of the neuroendocrine cell marker 7B2 in human ACTH secreting tumours. Clin. Endocrinol. **36**, 597–603

152 Lloyd, R. V., Jin, L., Qian, X., Scheithauer, B. W., Young, W. F. J. and Davis, D. H. (1995) Analysis of the chromogranin A post-translational cleavage product pancreastatin and the prohormone convertases PC2 and PC3 in normal and neoplastic human pituitaries. Am. J. Pathol. **146**, 1188–1198

153 Takumi, I., Steiner, D. F., Sanno, N., Teramoto, A. and Osamura, R. Y. (1998) Localization of prohormone convertases 1/3 and 2 in the human pituitary gland and pituitary adenomas: analysis by immunohistochemistry, immunoelectron microscopy, and laser scanning microscopy. Mod. Pathol. **11**, 232–238

154 Tomita, T. (2000) Immunocytochemical localization of prohormone convertase 1/3 and 2 in thyroid C-cells and medullary thyroid carcinoma. Clin. Res. **11**, 165–172

155 Konoshita, T., Gasc, J. M., Villard, E., Seidah, N. G., Corvol, P. and Pinet, F. (1994) Co-expression of PC2 and proenkephalin in human tumoral adrenal medullary tissues. Biochimie **76**, 241–244

156 Konoshita, T., Gasc, J. M., Villard, E., Takeda, R., Seidah, N. G., Corvol, P. and Pinet, F. (1994) Expression of PC2 and PC1/PC3 in human pheochromocytomas. Mol. Cell. Endocrinol. **99**, 307–314

157 Mbikay, M., Sirois, F., Yao, J., Seidah, N. G. and Chrétien, M. (1997) Comparative analysis of expression of the proprotein convertases furin, PACE4, PC1 and PC2 in human lung tumours. Br. J. Cancer **75**, 1509–1514

158 Rounseville, M. P. and Davis, T. P. (2000) Prohormone convertase and autocrine growth factor mRNAs are coexpressed in small cell lung carcinoma. J. Mol. Endocrinol. **25**, 121–128

159 Azzoni, C., D'Adda, T., Tamburrano, G., Coscelli, C., Madsen, O. D., Scopsi, L. and Bordi, C. (1998) Functioning human insulinomas. An immunohistochemical analysis of intracellular insulin processing. Virchows Arch. **433**, 495–504

160 Rockader, M., Jin, L., Zhang, S. and Lloyd, R. V. (2000) Insulin and glucagon mRNA expression and prohormone convertase immunoreactivity in normal and neoplastic pancreatic endocrine tissue. Endocr. Pathol. **11**, 179–183

161 Kimura, N., Pilichowska, M., Okamoto, H., Kimura, I. and Aunis, D. (2000) Immunohistochemical expression of chromogranins A and B, prohormone convertases 2 and 3, and amidating enzyme in carcinoid tumors and pancreatic endocrine tumors. Mod. Pathol. **13**, 140–146

162 Kajiwara, H., Itoh, Y., Itoh, J., Yasuda, M. and Osamura, R. Y. (1999) Immunohistochemical expressions of prohormone convertase (PC)1/3 and PC2 in carcinoids of various organs. Tokai J. Exp. Clin. Med. **24**, 13–20

163 Kimura, N., Ishikawa, T., Sasaki, Y., Sasano, N., Onodera, K., Shimizu, Y., Kimura, I., Steiner, D. F. and Nagura, H. (1996) Expression of prohormone convertase, PC2, in adrenocorticotropin-producing thymic carcinoid with elevated plasma corticotropin-releasing hormone. J. Clin. Endocrinol. Metab. **81**, 390–395

164 Cassidy, S. B. (1997) Prader-Willi syndrome. J. Med. Genet. **34**, 917–923

165 Gabreëls, B. A., Swaab, D. F., Seidah, N. G., van Duijnhoven, H. L., Martens, G. J. and van Leeuwen, F. W. (1994) Differential expression of the neuroendocrine polypeptide 7B2 in hypothalami of Prader-(Labhart)-Willi syndrome patients. Brain Res. **657**, 281–293

166 Gabreëls, B. A., Swaab, D. F., de Kleijn, D. P., Seidah, N. G., Van de Loo, J. W., van de Ven, W. J., Martens, G. J. and van Leeuwen, F. W. (1998) Attenuation of the polypeptide 7B2, prohormone convertase PC2, and vasopressin in the hypothalamus of some Prader-Willi patients: indications for a processing defect. J. Clin. Endocrinol. Metab. **83**, 591–599

167 Hendrick, J. P. and Hartl, F. U. (1993) Molecular chaperone functions of heat-shock proteins. Annu. Rev. Biochem. **62**, 349–384

168 Gething, M. J. (1999) Role and regulation of the ER chaperone BiP. Semin. Cell. Dev. Biol. **10**, 465–472

169 Shinde, U. and Inouye, M. (1996) Propeptide-mediated folding in subtilisin: the intramolecular chaperone concept. Adv. Exp. Med. Biol. **379**, 147–154

170 Anderson, E. D., VanSlyke, J. K., Thulin, C. D., Jean, F. and Thomas, G. (1997) Activation of the furin endoprotease is a multiple-step process: requirements for acidification and internal propeptide cleavage. EMBO J. **16**, 1508–1518

171 Fricker, L. D., McKinzie, A. A., Sun, J., Curran, E., Qian, Y., Yan, L., Patterson, S. D., Courchesne, P. L., Richards, B., Levin, N. et al. (2000) Identification and characterization of proSAAS, a granin-like neuroendocrine peptide precursor that inhibits prohormone processing. J. Neurosci. **20**, 639–648

172 Cameron, A., Fortenberry, Y. and Lindberg, I. (2000) The SAAS granin exhibits structural and functional homology to 7B2 and contains a highly potent hexapeptide inhibitor of PC1. FEBS Lett. **473**, 135–138

173 Qian, Y., Devi, L. A., Mzhavia, N., Munzer, S., Seidah, N. G. and Fricker, L. D. (2000) The C-terminal region of proSAAS is a potent inhibitor of prohormone convertase 1. J. Biol. Chem. **275**, 23596–23601