tion atrophy in small bowel: dissociation from endogenous gastrin levels. Gastroenterology *72* (1977) 890–895.
76 Peto, R., Epidemiology, multistage models and short term mutagenicity tests. Cold Spring Harbor Laboratories, 1977.
77 Polak, J. M., Ghatei, M. A., Wharton, J., Bishop, A. E., Bloom, S. R., Solcia, E., Brown, M. R., and Pearse, A. G. E., Bombesin-like immunoreactivity in the gastrointestinal tract, lung and central nervous system. Scand. J. Gastroent. *13*, suppl. 49 (1978) 148.
78 Robinson, J. W. L., Dowling, R. H., and Reichen, E. O., Eds, Intestinal Adaptation. II. MTP Press, Lancaster 1982.
79 Rozengurtz, E., and Sinnett-Smith, J., Characterisation of a gastrin releasing peptide from porcine non-antral gastrin tissue. Proc. natn. Acad. Sci. USA *80* (1983) 2936–2940.
80 Savage, A., Gornacz, G. E., Adrian, T. E., Goodlad, R. A., Wright, N. A., and Bloom, S. R., Elevation of PYY following intestinal resection in the rat is not responsible for the adaptive response. Gut *26* (1985) 1353–1358.
81 Sagor, G. R., Ghatei, M. A., Al-Mukhtar, M. Y. T., Wright, N. A., and Bloom, S. R., The effects of altered luminal nutrition on cellular proliferation and plasma concentrations of enteroglucagon after small bowel resection in the rat. Br. J. Surg. *69* (1982) 14–18.
82 Sagor, G. R., Ghatei, M. A., Al-Mukhtar, M. Y. T., Wright, N. A., and Bloom, S. R., Evidence for a humoural mechanism after intestinal resection, exclusion of gastrin but not enteroglucagon. Gastroenterology *54* (1983) 902–916.
83 Sagor, G. R., Ghatei, M. A., O'Shaughnessy, D. J., Al-Mukhtar, M. Y. T., Wright, N. A., and Bloom, S. R., Influence of somatostatin and bombesin on plasma enteroglucagon and cell proliferation after intestinal resection in the rat. Gut *26* (1985) 89–94.
84 Schwartz, M. A., and Storozuk, R. B., Enhancement of small intestine absorption by intraluminal gastrin. Gastroenterology *88* (1985) 1578.
85 Schieving, L. A., Yeh, Y. C., and Scheiving, L. E., Circadian phase-dependant stimulatory effects of epidermal growth factor on deoxyribonucleic acid synthesis in the tongue, oesophagus, and stomach of the adult male mouse. Endocrinology *105* (1979) 1475–1480.
86 Schieving, L. A., Yeh, Y. C., Tsai, T. H., and Scheiving, L. E., Circadian phase-dependant stimulatory effects of epidermal growth factor on deoxyribonucleic acid synthesis in the duodenum, jejunum, ileum, caecum, colon and rectum of the adult male mouse. Endocrinology *106* (1980) 1498–1503.
87 Smith, J., Cook, E., and Fotheringham, I., Chemical synthesis and cloning of a gene for human B-urogastrone. Nucl. Acids Res. *10* (1982) 4467–4482.
88 Solomon, T. E., Trophic effects of pentagastrin on gastrointestinal tract in fed and fasted rats. Gastroenterology *91* (1986) 108–116.
89 St. Hilaire, R. J., Gospodarowicz, D., and Kim, Y. S., Epidermal growth factor: effect on the growth of a human colon adenocarcinoma cell line. Gastroenterology *78* (1981) 1271.
90 Sukuki, T., Nakaya, M., Itoh, Z., Tatemoto, K., and Mutt, V., Inhibition of interdigestive contractile activity in the stomach by peptide YY in Heidenhain pouch dogs. Gastroenterology *85* (1983) 114–121.
91 Tam, J. P., Physiological effects of transforming growth factor in newborn mouse. Science *229* (1985) 673–675.
92 Tatemoto, K., and Mutt, V., Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. Nature *285* (1980) 417–418.
93 Thornburg, W., Matrisian, L., Magun, B., and Koldovsky, O., Gastrointestinal absorption of epidermal growth factor in suckling rats. Am. J. Physiol. *246* (1984) G80–G85.
94 Ulshen, M. H., Lyn-Cook, L. E., and Raasch, R. H., Effects of intraluminal epidermal growth factor on mucosal proliferation in the small intestine of adult rats. Gastroenterology *91* (1986) 1134–1140.
95 Varner, A. A., Modlin, I. M., and Walsh, J. H., High potency of bombesin for stimulation of human gastrin release and gastric acid secretion. Reg. Peptides *1* (1981) 289.
96 Voyles, N. R., Awoke, S., Wade, A., Bhathena, S. J., Smith, S. S., and Recant, L., Starvation increases gastrointestinal somatostatin in normal and obese Zuker rats: a possible regulatory mechanism. Horm. Metab. Res. *14* (1982) 392–395.
97 Walker-Smith, J. A., Phillips, A. D., Walford, N., Gregory, H., Fitzgerald, J. D., MacCullagh, K., and Wright, N. A., Intravenous epidermal growth factor/urogastrone increases small intestinal cell proliferation in congenital microvillous atrophy. Lancet *2* (1985) 1239–1240.
98 Wharton, J., Polak, J. M., Bloom, S. R., Ghatei, M. A., Solcia, E., Brown, M. R., and Pearse, A. G. E., Bombesin-like immunoreactivity in the lung. Nature *273* (1978) 769.
99 Willems, G., Cell renewal in the gastric mucosa. Digestion *6* (1972) 46–63.
100 Williamson, R., Intestinal adaptation. 2. Mechanisms of control. N. Engl. J. Med. *298* (1978) 1444–1450.
101 Wright, N. A., Regulation of growth by peptides, in: Gut Hormones. Eds S. R. Bloom and J. M. Polak. Churchill Livingstone, Edinburgh 1981.
102 Wright, N. A., and Alison, M., The Biology of Epithelial Cell Populations, vol. 1. Clarendon Press, Oxford 1984.
103 Wright, N. A., and Alison, M., The Biology of Epithelial Cell Populations, vol. 2. Clarendon Press, Oxford 1984.

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Precursors to regulatory peptides: their proteolytic processing

by P. C. Andrews, K. Brayton and J. E. Dixon

Department of Biochemistry, Purdue University, West Lafayette (Indiana 47907, USA)

Summary. Precursors to regulatory peptides undergo maturation processes which include proteolytic processing. The enzymes involved in this process remove the hydrophobic peptide located at the amino-terminus of the precursor. Endoprotease cleavage also occurs at single and two adjacent basic residues, this is followed by a removal of basic residues located at the C-terminus of the peptides by a carboxypeptidase-like enzyme.
Key words. Prohormone processing; regulatory peptides; precursors; proteolytic enzymes.

Regulatory peptides are diverse in their function and localization; however, they share a common property in that they all are initially synthesized as larger precursors which are processed proteolytically to form biologically active products[15,45]. Figure 1 is a schematic representation of several regulatory peptide precursors showing their processing sites and indicating that these precursors can have a molecular weight greater than 10 times that of the biologically active peptides[29], or, they may lose only a few amino acids during their maturation process[63].

Precursors to regulatory peptides have in common 1) a similar 'route' from their site of synthesis to the ultimate export of their products from the cell and 2) they all undergo proteolytic processing events. The proteolytic processing events include removal of the signal sequence, which is necessary for sequestration of the protein into the endoplasmic reticulum as well as subsequent endoproteolytic and exoproteolytic cleavages. Specific regulatory peptides can also undergo other post-translational modifications which include disulphide bond formation, carbohydrate addition, sulphation, phosphorylation, acetylation, and amidation to mention only a few of the numerous modifications which have been described[87].

In this brief review, it is not possible to examine throughly all

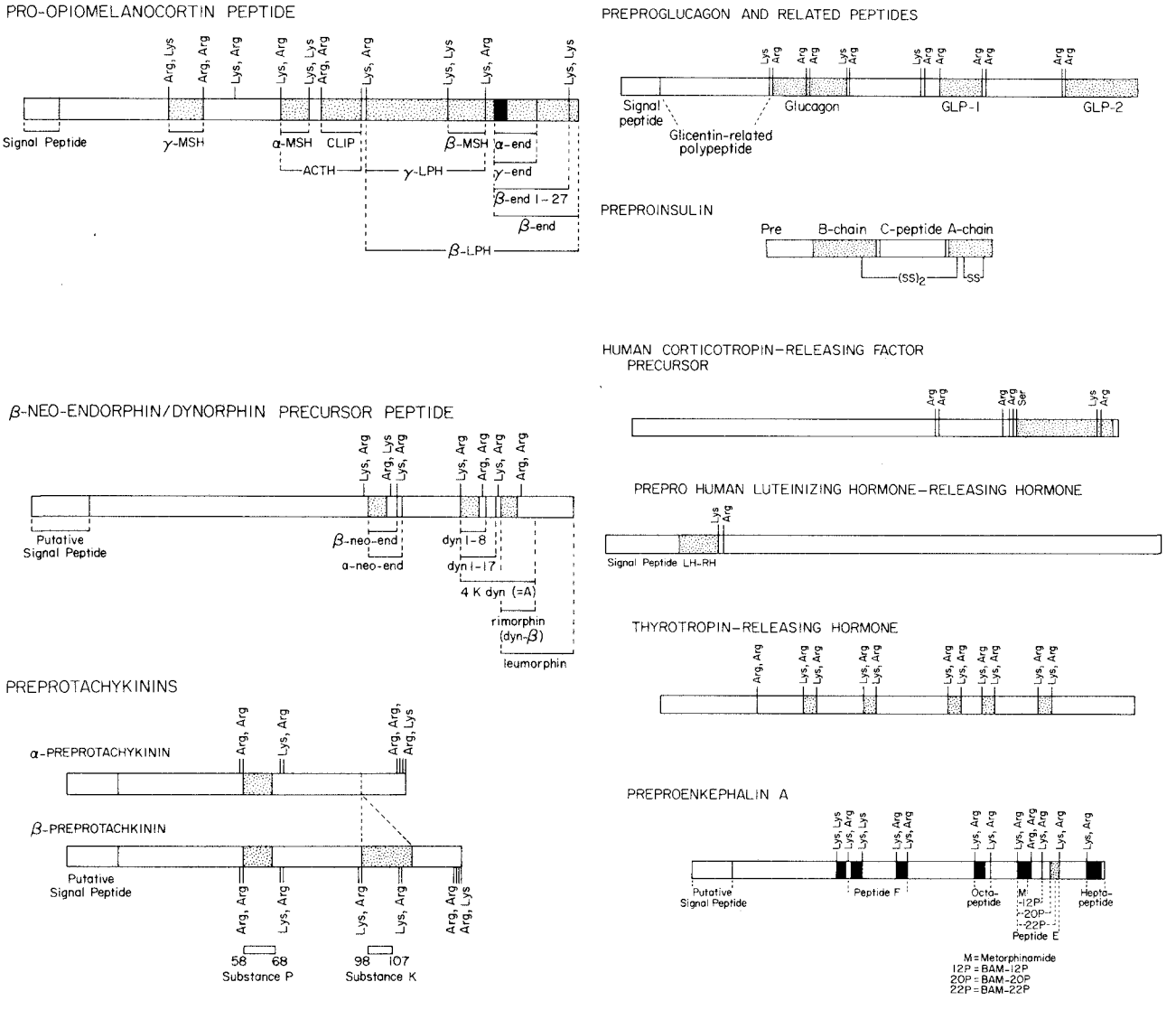

Figure 1. Schematic representation of peptide precursors. Stippled areas indicate biologically important products; Black indicates methionine enkephalin. Data sources are as follows: Proglucagon[4]; Preproinsulin[5]; Corticotrophin-releasing factor precursor[70]; Prepro LH-RH precursor[69]; Thyrotropin-releasing hormone[43]; Prepro-enkephalin A[44]; Prepro-opiomelanocortin[49]; Preproenkephalin B[19,35]; Preprotachykinins[56].

aspects of post-translational modifications of precursors to regulatory peptides. We will instead, focus on one of the events that regulatory peptides have in common, namely, the proteolytic processing of the precursors to form their corresponding regulatory peptides.

Biosynthesis of precursors to regulatory peptides. The nomenclature used to identify the specific intermediates observed during protein biosynthesis arose from the early work of Steiner and his colleagues[73] who identified a large precursor form of insulin which they referred to as proinsulin. A precursor to proinsulin was observed in cell-free translation experiments, the larger precursor was referred to as preproinsulin[74] (fig. 1). This terminology has generally been applied to the various intermediates of regulatory peptide biosynthesis.

The signal peptide

Proteins destined for secretion from the cell are initially synthesized in the cytoplasm, but become tightly bound to the endoplasmic reticulum as synthesis proceeds. The generally accepted model proposed by Blobel and Dobberstein in 1975 for protein export from the cell has been referred to as the signal hypothesis[7,8]. This model suggests that the initial synthesis of the growing peptide chain in the cytoplasm results in attachment of the amino terminus of the exported protein to membranes of the endoplasmic reticulum[81]. The interactions between the endoplasmic reticulum and growing peptide-ribosomal complex are complicated, but they clearly involve protein complexes such as the signal recognition particle, which is composed of six proteins and a 7S RNA molecule[82-85]. In addition, other proteins appear to play important roles in this interaction[85].

All precursors to regulatory peptides which are transported via this vectorial mechanism into the endoplasmic reticulum, harbor an amino terminal sequence of approximately 16–30 amino acid residues which generally includes 4–12 hydrophobic residues. This amino acid sequence has been referred to as the 'signal sequence' because it appears to contain the

'code' which designates that this protein is to be routed to the endoplasmic reticulum[7,8].

Signal sequences are not normally observed on newly synthesized proteins found within cells. This suggests that the signal sequence is rapidly removed from the precursor, while the growing peptide is still attached to the ribosome. In order to observe the signal sequence, it is necessary to carry out cell-free translation experiments with isolated messenger RNAs. In the absence of membranes from endoplasmic reticulum, the signal sequence is not removed and can be readily identified following isolation of the regulatory peptide precursor. An example of a signal sequence identified on a preproregulatory peptide was recently described by Minth et al.[54]. RNA isolated from a human pheochromocytoma was translated in a cell-free translation system with radioactive leucine or methionine. Minth et al.[54] isolated the precursor by immunoprecipitation with antibodies directed towards the regulatory peptide, in this instance an antibody produced against neuropeptide Y. The antibodies recognized the larger precursor (preproneuropeptide Y) and selectively precipitated the product. The results of the cell-free translation and immunoprecipitation of the precursor are illustrated in figure 2.

The immunoprecipitated product was subjected to automated Edman degradation. This results in selective removal of amino acids from the amino terminus. When the radioactivity in each cycle of the Edman degradation is plotted against the amino acid sequence deduced from the cDNA sequence one can see that there is exact agreement between the [^{3}H] leucine in cycles 2, 7, 9, 12, 14, 16, 18, 19, 22, 25, and the locations of the deduced leucine residues. The only cycle in which [^{35}S] methionine is seen is cycle 1. The results of the sequencing reactions and the deduced amino acid sequence of the signal peptide of preproneuropeptide Y are shown in figure 3. The signal sequence of preproneuropeptide Y is removed as a result of proteolysis between Ala-28 and Tyr-29. Some of the factors which govern the removal of the signal sequence from secretory proteins are now under study.

Cleavage of the signal peptide

Both eukaryotic and prokaryotic cells appear to use similar mechanisms for protein export. Bacterial cells have been shown to be capable of exporting various eukaryotic proteins[22,78] and conversely, eukaryotic cells will secrete certain bacterial proteins[86]. Much of our knowledge concerning the protease responsible for cleavage of the signal sequence from the growing nascent protein comes from studies by Wickner and his colleagues on the biogenesis of the M13 procoat protein[65]. The protease which cleaves the signal peptide from the M13 protein has been isolated and characterized. It appears to be an integral membrane protein of 37,000 daltons[88]. This peptidase isolated from bacteria seems to be quite homologous to the signal peptidase of the eukaryotic endoplasmic reticulum[65]. For example, the bacterial enzyme will cleave preproproteins such as the honeybee prepromellitin, human-prepro placental lactogen and preproinsulin[65]. The specific site of cleavage of the signal peptide in numerous regulatory peptide precursors has been established. No specific 'sequence' of amino acids would appear to be responsible for recognition by the signal peptidase; rather, higher order structure, (i.e. secondary structure) seems to play an important role in determining the exact site of cleavage. Inouye and colleagues[36,37,79,80] have examined the structure-function relationship of residues located within the signal sequence. The importance of the central hydrophobic region is readily apparent from a number of reports which describe secretion-defective mutants with substitutions in this region[3,16,17,53]. In addition, using site-directed mutagenesis, Inouye et al. showed that selective alterations of amino acids within the hydrophobic segment have dramatic effects upon the secretory potential for the signal sequence[37]. Substitution of amino acids at or near the cleavage site can result in either the absence of processing or alternate processing at new sites. The analysis of the conformation required for the cleavage of prolipoprotein suggest that the residues near the cleavage site are probably in the β-turn structure. Substitution of residues which reduce the β-turn potential seem to alter dramatically the ability of the signal peptidase to carry out effective cleavage of the precursor sequence.

Cleavage at two adjacent basic residues

As discussed in the previous section, all regulatory peptides to date appear to be synthesized initially as preproproteins with rapid removal of the signal peptide. The resulting propeptides can be proteolytically processed at either single basic residues or at two adjacent basic residues. Arginine rather than lysine seems to be preferred in the P_1 position of basic dipeptides[75]. Figure 1 shows a number of examples of proregulatory proteins which harbor these processing sites.

The mammalian enzyme which recognizes and cleaves the prohormone at the two basic residues has not been isolated in pure form, nor characterized. The unicellular eukaryote *Saccharomyces cerevisiae* produces precursors for at least two secreted biologically active peptides, prepro-α-factor and

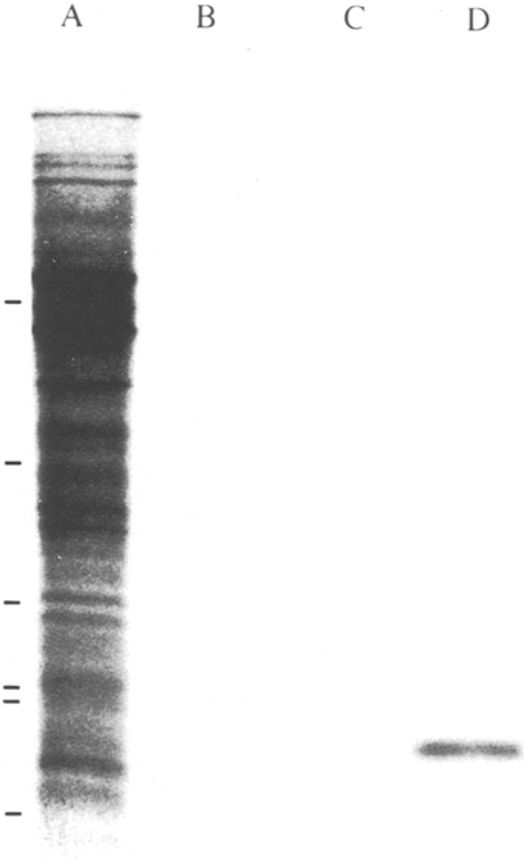

Figure 2. Immunoprecipitation of translation products directed by human pheochromocytoma RNA. Translations were carried out in a wheat germ cell-free translation system and then analyzed on sodium dodecylsulphate/15% polyacrylamide gels. Lanes: A, total translation products (one-tenth of reaction); B, precipitation with preimmune serum; C, precipitation with anti-NPY serum (YN-12) in the presence of 5 μg of porcine NPY; D, precipitation with anti-NPY (YN-12). Protein standards (in daltons) from top to bottom are ovalbumin (43,000) α-chymotrypsinogen (25,700), β-lactoglobulin (17,400), lysozyme (14,300), cytochrome c (12,300), and bovine trypsin inhibitor (6200).

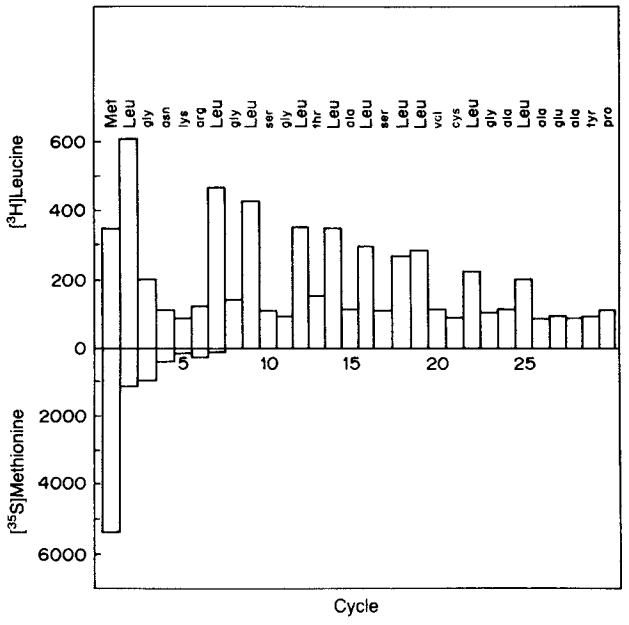

Figure 3. The NH_2-terminal sequence analysis of prepro-NPY. Sequential Edman degradation was carried out on the in vitro cell-free translation product immunoprecipitated with antiserum directed against porcine NPY. The radioactivity corresponding to [^{35}S] methionine and [^{3}H] leucine was determined for 30 cycles. The positions of the radiolabeled amino acids in the immunoprecipitated translation product are shown along with the amino acid sequence determined from nucleotide sequence analysis. The positions of Leu and Met are capitalized.

preprokiller toxin which contain pairs of basic residues which are presumptive processing sites. In KEX 2 mutant yeast cells neither of these proteins is produced in their mature form. The KEX 2 mutation in yeast resides in a gene that encodes a novel endoprotease specific for cleaving these two substrates on the carboxyl side of pairs of basic residues[38]. The KEX 2 gene has been cloned and introduced into yeast cells on multicopy plasmids such that the KEX 2 protease is overproduced about 30-fold. From these cells the KEX 2 endoprotease has been purified about 100-fold, and the following catalytic properties have been elucidated. The membrane bound enzyme has a neutral pH optimum, and the enzyme displays a marked substrate preference. The relative rates of hydrolysis of peptide bonds are shown below.

$$\text{-ArgArg}\overset{\downarrow}{-} \cong \text{-LysArg}\overset{\downarrow}{-} \gg \text{-LysLys}\overset{\downarrow}{-} \cong \text{-Arg}\overset{\downarrow}{-} \gg \text{Lys}\overset{\downarrow}{-}$$

The enzyme also requires an active site thiol, as determined by its susceptibility to thiol inhibitors and is unaffected by several classical serine protease inhibitors. The most potent enzyme inhibitor found to date is a reagent originally prepared by Kettner and Shaw (1981), alanyl-lysyl-arginyl-chloromethylketone. The KEX 2 endoprotease can be covalently tagged and radioactively labeled by a derivative of this inhibitor, which will do the same for cathepsin B. The activity of this enzyme also appears to be completely dependent on the presence of Ca^{++} ions[28].

The yeast KEX 2 endoprotease resembles the mammalian proteases called calpains. The calpains are Ca^{++} dependent neutral thiol proteases[13,55]. In experiments using synthetic fluorogenic substrates to determine their substrate specificity, it was found that cleavage occurred on the carboxyl side of a Tyr, Met or Arg residue provided that preceding residue is hydrophobic[67]. Although many proteins have been examined as substrates of calpain cleavage, the true biological substrates of the calpains has not been determined. It has not been easy to distinguish the 'prohormone' processing enzyme(s) from other proteases found in lysozymes. Studies which have examined the conversion of proinsulin, proglucagon and prosomatostatin I to their corresponding mature hormones in the secretory granules of anglerfish pancreatic islets found that at least one of the enzymes involved in the conversion of these prohormones was different from other intracellular proteases. The enzyme was shown to be a thiol protease with a pH optimum near 5, cleaves at pairs of basic amino acids and may possibly require the presence of segments of the prohormone for proper substrate recognition and/or binding[20,21,57]. It is associated with the membrane of the secretory granule. The enzyme is similar to cathepsin B, but has a more restricted substrate specificity and is not inhibited by N-p-tosyl-L-lysine chloromethylketone. This may be the enzyme with 'trypsin like' substrate specificity originally proposed for the first step in insulin processing[39-41,76,90,91].

Recently, a 70 kda glycoprotein has been purified from secretory vesicles of the intermediate lobe[46] and the neural lobe[61] of the bovine pituitary. The activity assay involved conversion of mouse proopiomelanocortin to 21 kDa ACTH,

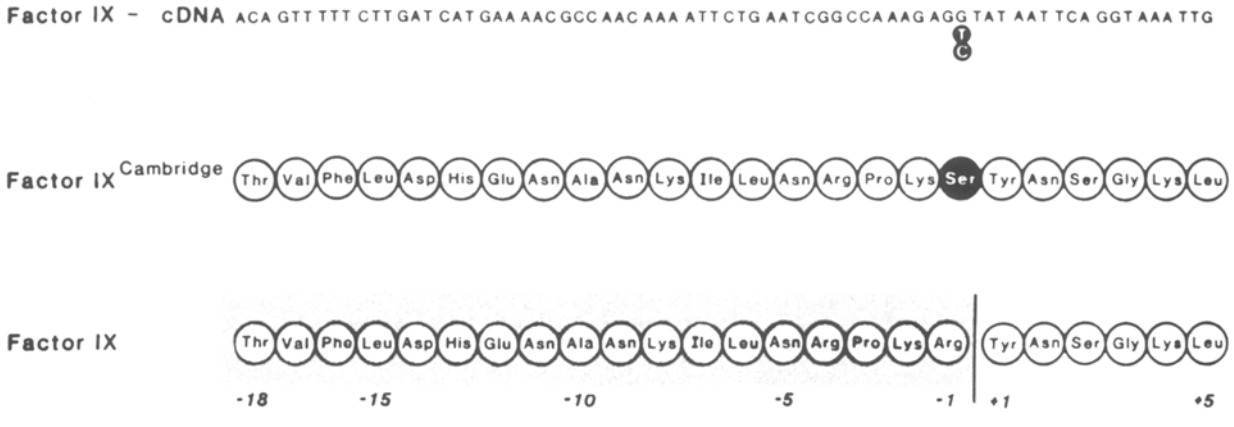

Figure 4. Amino acid sequence of factor $IX^{Cambridge}$. Factor $IX^{Cambridge}$ contains an 18-residue NH_2-terminal extension homologous to the predicted amino acid sequence of the propeptide of factor IX (stippled) extrapolated from the known cDNA sequence of factor IX[12,42]. Factor IX has an NH_2-terminal sequence of Tyr-Asn-Ser-. Residue 18 of factor $IX^{Cambridge}$, analogous to the arginine at residue −1 of the factor IX precursor, is mutated to a serine (solid). This point mutation ($Arg^{-1} \rightarrow Ser^{-1}$) in the factor IX propeptide precludes normal proteolytic processing between residues −1 and +1. The codon for arginine-1 in the factor IX precursor, AGG, is altered to either AGT or AGC (solid), codons that code for serine. Reproduced from Diuguid et al.[14].

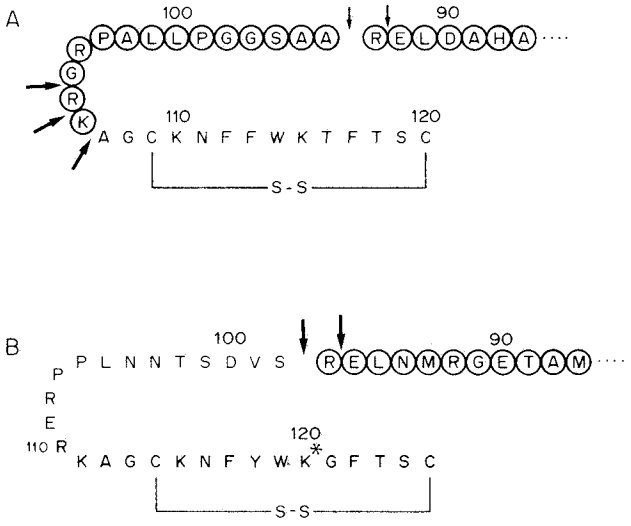

Figure 5. Processing sites for *A* anglerfish preprosomatostatin I and *B* anglerfish preprosomatostatin II. The residues belonging to the non-somatostatin portion of prosomatostatin are circled. The residues are numbered from the initiator methionine. The asterisk indicates the site of lysyl hydroxylation. The peptide bonds cleaved during conversion to the mature hormones are indicated by large arrows. Small arrows indicate minor processing sites.

βLPH, and 23 kDa ACTH. The enzymes have acidic pH optima and the enzyme from the intermediate lobe cleaves on the carboxyl side of Arg in the Lys-Arg dipeptide when provasopressin is the substrate to produce arginine vasopressin extended at the carboxyl terminus by Gly-Lys-Arg. The Gly residue contributes the carboxyl amide during later steps of processing. The enzymes from both sources were not inhibited by diisopropyl-fluorophosphate, p-chloromercuribenzoate, or EDTA, but were inhibited by pepstatin A, an inhibitor of aspartyl proteinases. Because the enzymes from both sources are very similar in physical properties and in substrate specificities, the possibility exists that the enzymes from both sources are identical or are closely related. Although the enzymes described by Loh and colleagues will carry out conversion of several prohormones and are located in secretory vesicles, experiments unambiguously demonstrating their requirement for prohormone conversion in vivo are unattainable until genetic manipulation of the activity becomes feasible.

A prohormone 'converting enzyme' activity described by Noe and his colleagues appears to be membrane associated and is found in microsomes and secretory granules[58]. This membrane association of converting enzymes has been demonstrated in rat anterior pituitary[11], neurointermediate lobe[47], and rat hypothalamic synaptosomes[89]. It has been proposed that newly synthesized islet prohormones are membrane associated in the microsome and secretory granule and that the RER/Golgi complex and secretory granule membranes serve as a matrix to bring the enzyme and substrate together[58].

The importance of processing at adjacent basic residues

Although the mammalian enzyme which selectively cleaves at two adjacent basic amino acid residues has not been characterized in detail, the importance of these dibasic sites for the proper processing of prohormones has been noted in several instances. Families with hyperinsulinemia have been described[66,71]. Normally, proinsulin is cleaved between Arg-65 and Gly-66, a peptide bond which connects the C-peptide and A chain of proinsulin (fig. 1). A point mutation in the Arg-65 codon changes this residue to a histidine which results in a blockage of the post-translational cleavage of proinsulin to insulin[71]. Alterations in the amino acid sequence of proalbumin can also result in variants which do not undergo conversion to albumin[1,9]. Normally proalbumin is processed at two adjacent arginine residues. In one of the variants, the alteration results in an Arg-Glu substitution while in another variant it causes a His-Arg substitution. These changes result in unprocessed proalbumin. Recently a variant of factor IX coagulant activity was described which resulted in hemophilia B[6,14]. Factor IX Cambridge has a Lys-Ser substitution for the normally observed post-translational processing site Lys-Arg (fig. 4). This point mutation in a human protein precursor impairs proteolytic processing and results in a disease state (hemophilia B).

Proteolytic cleavage at single basic residues and processing by exopeptidases

Flexibility in proteolytic processing is important for tissue-specific processing of prohormones. For example, the major physiologically active peptide produced as a result of proglucagon (fig. 1) processing in the pancreas appears to be glucagon. A larger peptide derived from the remainder of proglucagon is also produced[62], but has not yet been shown to possess biological activity. In contrast, four different physio-

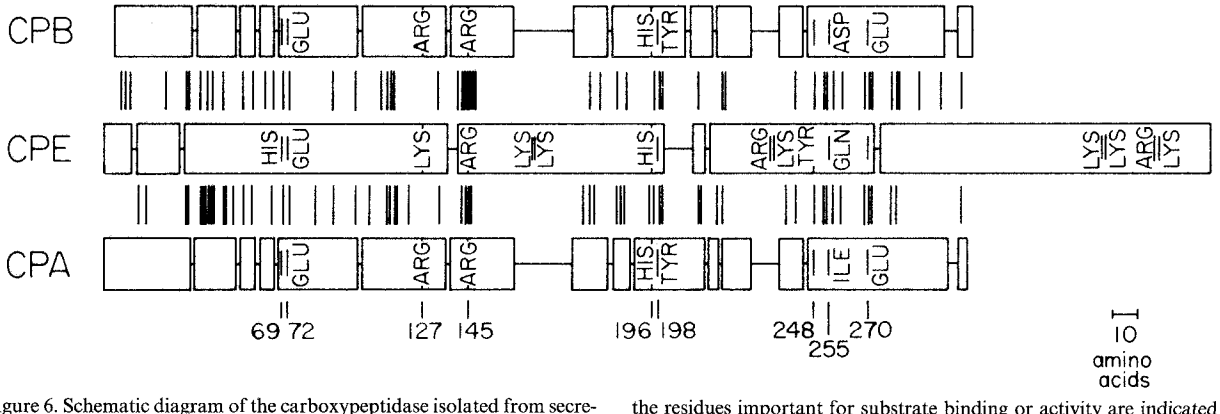

Figure 6. Schematic diagram of the carboxypeptidase isolated from secretory vesicles which is specific for basic residues. It is referred to as enkephalin converting enzyme (CPE) by Fricker and Snyder[24,25]. The stuctures of carboxypeptidases A and B (CPA and CPB, respectively) are also indicated for comparison. Deleted regions are indicated by a thin line and the residues important for substrate binding or activity are indicated by their three-letter codes. The His residues at position 69 in both CPA and CPB are not indicated but are present. Residues in CPA or CPB which are identical to those in CPE are indicated by lines in the spaces between the proteins.

logically active products[60] are produced in the gut (glicentin, oxyntomodulin, GLP-1, and GLP-2). One way of achieving this differential processing is through the use of multiple recognition sites for different converting enzymes.

Although prohormone conversion frequently occurs at basic dipeptides, a growing number of processing sites are being uncovered which involve cleavage at single arginine residues. One of these is a single Arg residue in proglucagon which is utilized during production of the GLP-1 mentioned above[32]. Other sites in proglucagon occur at basic dipeptides.

Another excellent example of the differential use of single versus double-basic conversion sites occurs in the processing of prosomatostatin (fig. 5) in mammals and in fish[2, 59, 72]. The processing of prosomatostatin in mammalian pancreas involves cleavage at an Arg-Lys dipeptide resulting in formation of a 14-residue form of somatostatin. In the gut, processing at an earlier, single Arg residue produces a 28-residue somatostatin[31, 19, 64] having different physiological activities[10, 50, 52]. Thus, two hormones or regulatory peptides may be obtained from a single precursor via use of different processing sites. The two forms of somatostatin are produced in separate cells in anglerfish pancreas[51]. The situation in the fish is complicated by the presence of two prosomatostatins, one of which is converted to the 14-residue somatostatin, and one which is converted to a 28-residue somatostatin (fig. 5). However, both forms contain equivalent monobasic and dibasic putative processing sites. The enzymes responsible for the recognition of the two different processing sites have been shown to be differentially localized in fish pancreas[58] and can be resolved chromatographically[48]. The activity which produces the 14-residue somatostatin from prosomatostatin-I (fig. 5) will not cleave prosomatostatin-II[48] even though the gene II product also contains an equivalent basic dipeptide immediately before the cryptic 14-residue somatostatin.

The protease(s) responsible for processing at single arginine residues has not yet been isolated. However, from the known structures of the substrates and products of processing some of the parameters important for substrate-specificity may be deduced. Although a given prohormone may contain several single arginine residues, only a limited number are utilized. Inspection of cleavage sites containing single residues reveals no general similarities in primary sequences, although some of the cleavage sites may be grouped into those having nearby proline residues[68]. However, not all arginine residues in close proximity to proline are cleaved. The lack of obvious sequence homology among processing sites suggests that the processing enzyme(s) recognizes some component of secondary and/or tertiary structure at the cleavage site in addition to the single arginine residue.

Both classes of endopeptidases with specificity directed either towards single or to adjacent basic residues produce products having terminal basic residues. In many instances these residues must be removed to produce the active form of the peptide. Secretory vesicles have been reported to contain both an aminopeptidase[30] and a carboxypeptidase[23–25, 33, 34, 77] with specificities directed toward basic residues.

The potent carboxypeptidase has been reported from a wide range of endocrine tissues. The enzyme is a metallopeptidase and has an acidic pH optimum in the range 5.0–6.0[24, 25, 34, 77]. The carboxypeptidase from secretory granules is activated by Co^{2+} and can be distinguished from the lysosomal enzyme which is not. The secretory granule carboxypeptidase can be inhibited by chelating agents and by active-site directed inhibitors such as guanidinoethyl-mercaptosuccinic acid[24]. Two forms of the enzyme exist, a membrane-bound form and a soluble form having molecular weights of approximately 52,000 and 50,000 daltons respectively[24, 25, 77]. The soluble form has been purified to homogeneity[26] and the cDNA has been cloned and sequenced[27]. The deduced protein sequence indicates some homology (fig. 6) with carboxypeptidases A and B (20% and 17% respectively). The homology between carboxypeptidases A and B is 48%, suggesting that although the carboxypeptidases are related, A and B diverged from one another more recently than the secretory granule carboxypeptidase. Major differences between the converting enzyme and carboxypeptidases A and B include a carboxyl-terminal extension of 120 amino acids and changes in the substrate-binding regions (fig. 6).

An aminopeptidase has also been reported from secretory vesicles[30]. The enzyme is a membrane-bound metallopeptidase having a broad pH optimum in the range 5.5–7.5. Although the enzyme is specific for amino-terminal basic residues, its involvement in prohormone conversion has not yet been established.

Note added: Recently, Evans et al. (Proc. natn. Acad. Sci. USA *83* (1986) 581) reported the purification to near homogeneity from canine pancreatic microsomes, the eukaryotic signal peptidase. In contrast to the prokaryotic protease, the purified canine enzyme consists of a complex of six polypeptides with apparent molecular masses of 25, 23, 22, 18 and 12 kDa. The 22 and 23 kDa proteins appear to be glycoproteins, however, only one of the subunits appears to function in signal peptide cleavage.

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1 Abdo, Y., Rousseaux, J., and Dautrevaux, M., FEBS Lett. *131* (1981) 286.
2 Andrews, P.C., Hawke, D., Shively, J.E., and Dixon, J.E., J. biol. Chem. *259* (1984) 15021.
3 Bedouelle, H., Bassford, P.J., Fowler, A.V., Zabin, I., Beckwith, J., and Hofnung, M., Nature *285* (1980) 78.
4 Bell, G.I., Sanchez-Pescador, R., Laybourn, P.J., and Najarian, R.C., Nature *304* (1983) 368.
5 Bell, G.I., Swain, W.F., Pictet, R., Cordell, B., Goodman, H.M., and Rutter, W.J., Nature *282* (1979) 525.
6 Bentley, A.K., Rees, D.J.G., Rizza, C., and Brownlee, G.G., Cell *45 (1986)* 343.
7 Blobel, G., and Dobberstein, B., J. Cell Biol. *67* (1975) 835.
8 Blodel, G., and Dobberstein, B., J. Cell Biol. *67* (1975) 852.
9 Brennan, S.O., and Carell, R.W., Nature, Lond. *274* (1978) 908.
10 Brown, M., Rivier, J., and Vale, W., Endocrinology *108* (1981) 2391.
11 Chang, T.-L., and Loh, Y.P., Endocrinology *112* (1983) 1832.
12 Choo, K.H., Gould, K.G., Rees, D.J.G., and Brownlee, G.G., Nature Lond. *299* (1982) 178.
13 DeMartino, G.N., and Croall, D.E., Biochemistry *22* (1983) 6287.
14 Diuguid, D.L., Rabiet, M.J., Furie, B.C., Liebman, H.A., and Furie, B., Proc. natn. Acad. Sci. USA *83* (1986) 5803.
15 Douglass, J., Civelli, O., and Herbert, E., A. Rev. Biochem. *53* (1984) 665.
16 Emr, S.D., Hedgpeth, J., Clement, J.M., Silhavy, T.J., and Hofnung, M., Nature *285* (1980) 82.
17 Emr, S.D., Schwartz, T.N., and Silhavy, T.J., Proc. natn. Acad. Sci. USA *75* (1978) 5802.
18 Esch, F., Böhlen, P., Ling, N., Benoit, R., Brazeau, P., and Guillemin, R., Proc. natn. Acad. Sci. USA *77* (1980) 6827.
19 Fischli, W., Goldstein, A., Hunkapiller, M.W., and Hood, L.E., Proc. natn. Acad. Sci. USA *79* (1982) 5435.
20 Fletcher, D.J., Noe, B.D., Bauer, G.E., and Quigley, J.P., Diabetes *29* (1980) 593.
21 Fletcher, D.J., Quigley, J.P., Bauer, G.E., and Noe, B.D., J. Cell Biol. *90* (1981) 312.
22 Fraser, T.H., and Bruce, B.J., Proc. natn. Acad. Sci. USA *75* (1978) 5936.
23 Fricker, L.D., Plummer, T.H., and Snyder, S.H., Biochem. biophys. Res. Commun *111* (1983) 994.
24 Fricker, L.D., and Snyder, S.H., Proc. natn. Acad. Sci. USA *79* (1982) 3886.
25 Fricker, L.D., Suppattapone, S., and Snyder, S.H., Life Sci. *31* (1982) 1841.

26 Fricker, L.D., and Snyder, S.H., J. biol. Chem. *258* (1983) 10950.
27 Fricker, L.D., Evans, C.J., Esch, F.S., and Herbert, E., Nature *323* (1986) 461.
28 Fuller, R.S., Brake, A.J., Julius, D.J., and Thorner, J. in: Protein Transport and Secretion, p. 97 Ed. M.-J. Gething. Cold Spring Harbor Press 1985.
29 Funckes, C.L., Minth, C.D., Deschenes, R., Magazin, M., Tavianini, M.A., Sheets, M., Collier, K., Weith, H.L., Aron, D., Roos, B.A., and Dixon, J.E., J. biol. Chem. *258* (1983) 8781.
30 Gainer, H., Russell, J.T., and Loh, Y.P., FEBS Lett. *175* (1984) 135.
31 Hobart, P., Crawford, R., Shen, L-P., Pictet, R., and Rutter, W.J., Nature *288* (1980) 137.
32 Holst, J.J. Ørskov, C., Schwarz, T.W., Buhl, T., and Baldissera, F.G.A., 22nd Annual Meeting of Eur. Assoc. for the Study of Diabetes; Abstract No. 217, Diabetologia *29* (1986) 549A.
33 Hook, V.Y.H., Eiden, L.E., and Brownstein, M.J., Nature *295* (1982) 341.
34 Hook, V.Y.H., and Loh, Y.P., Proc. natn. Acad. Sci. USA *81* (1984) 2776.
35 Horikawa, S., Takai, T., Toyosato, M., Takahashi, H., Noda, M., Kakidani, H., Kubo, T., Hirose, T., Inayama, S., Hayashida, H., Miyata, T., and Numa, S., Nature *306* (1983) 611.
36 Inouye, S., Soberon, X., Franceschini, T., Nakamura, K., Itakura, K., and Inouye, M., Proc. natn. Acad. Sci. USA *79* (1982) 3438.
37 Inouye, M., Inouye, S., Pollitt, S., Ghrayed, J., and Lunn, C.A., Structural and Functional Analysis of the Prolipoprotein Signal Peptide of *Escherichia coli,* in: Protein Transport and Secretion, p. 54. Ed. M.-J. Gething. Cold Spring Harbor Press 1985.
38 Julis, D., Brake, A., Blair, L., Kunisawa, R., and Thorner, J., Cell *37* (1984) 1075.
39 Kemmler, W., and Steiner D.F., Biochem. biophys. Res. Commun. *41 (1970)* 1223.
40 Kemmler, W., Peterson, J.D., and Steiner, D.F., J. biol. Chem. *246* (1971) 6786.
41 Kemmler, W., Steiner, D.F., and Borg, J., J. biol. Chem. *248* (1973) 4544.
42 Kurachi, K., and Davie, E.W., Proc. natn. Acad. Sci. USA *79* (1982) 6461.
43 Lechan, R.M., Wu, P., Jackson, I.M.D., Wolf, H., Cooperman, S., Mandel, G.E., and Goodman, R.H., Science *231* (1986) 159.
44 Lewis, R.V., Stern, A.S., Kimura, S., Rossier, J., Stein, S., and Udenfriend, S., Science *208* (1980) 1459.
45 Loh, Y.P., and Gainer, H., Biosynthesis and processing of neuropeptides, in: Brain Peptides, p. 79. Eds D.T. Krieger et al. John Wiley and Sons, New York 1983.
46 Loh, Y.P., Parish, D.C., and Tuteja, R., J.biol. Chem. *260* (1985) 7194.
47 Loh, Y.P., and Gainer, H., Proc. natn. Acad Sci. USA *79* (1982) 108.
48 Mackin, R.B., Ph. D. Dissertation, Dept. of Anatomy and Cell Biology, Emory University School of Medicine, 1987.
49 Mains, R.E., and Eipper, B.A., J. biol. Chem. *254* (1979) 7885.
50 Mandarino, L., Stenner, D., Blanchard, W., Nissen, S., Gerich, J., Ling, N., Brazeau, P., Bohlen, P., Esch, F., and Guillemin, R., Nature *291* (1981) 76.
51 McDonald, J.K., Greiner, F., Gauer, G.E., Elde, R.P., and Noe, B.D., J. Histochem. Biochem. *35* (1987) 155.
52 Meyers, C.A., Murphy, W.A., Redding, T.W., Coy, D.H., and Schally, A.V., Proc. natn. Acad. Sci. USA *77 (1980)* 6171.
53 Michaelis, S., Inouye, H., Oliver, D., and Beckwith, J., J. Bact. *154* (1983) 366.
54 Minth, C.D., Bloom, S.R., Polak, J.M., and Dixon, J.E., Proc. natn. Acad. Sci. *81* (1984) 4577.
55 Murachi, T., Intracellular Ca^{2+} protease and its inhibitor protein: calpain and calpastatin, in: Calcium and Cell Function, vol. 4, p. 377. Ed. W.Y. Cheung. Academic Press, New York 1983.
56 Nawa, H., Hirose, T., Takashima, H., Inayama, S., and Nakanishi, S., Nature *306* (1983) 32.
57 Noe, B.D., J. biol. Chem. *256* (1981) 4940.
58 Noe, B.D., Debo, G., and Spiess, J., J. Cell Biol. *99* (1984) 578.
59 Noe, B.D., Spiess, J., Rivier, J.E., and Vale, W., Endocrinology *105* (1979) 1410.
60 Ørskov, C., Holst, J.J., Knuhtsen, S., Baldissera, F.G.A., Poulsen, S.S., and Nielsen, O.V., Endocrinology *119* (1986) 1467.
61 Parish, D.C., Tuteja, R., Altstein, M., Gainer, H., and Loh, Y.P., J.biol. Chem. *261* (1986) 14392.
62 Patzelt, C., and Schiltz, E., Proc. natn. Acad. Sci. USA *81* (1984) 5007.
63 Potts, J.T.Jr, Kronenberg, H.M., Habener, J.F., and Rich, A., Ann. N.Y. Acad. Sci. *343* (1980) 38.
64 Pradayrol, L., Jörnvall, H., Mutt, V., and Ribet, A., FEBS Lett. *109* (1980) 55.
65 Rice, M.C., and Wickner, W.T., Mechanisms of membrane assembly and protein secretion in *Escherichia coli*, in: Protein Transport and Secretion, p. 44. Ed. M.-J. Gething. Cold Spring Harbor Press 1985.
66 Robbins, D.C., Blix, P.M., Rubenstein, A.H., Kanazawa, Y., Kosaka, K., Tager, H.S., Nature Lond. *291* (1981) 679.
67 Sasaki, T., Kikuchi, T., Yumoto, N., Yoshimura, N., and Murachi, T., J. biol. Chem. *259* (1984) 12489.
68 Schwartz, T.W., FEBS Lett. *200* (1986) 1.
69 Seeburg, P.H., and Adelman, J.P., Nature *311* (1984) 666.
70 Shibahara, S., Morimoto, Y., Furutani, Y., Notake, M., Takahashi, H., Shimizu, S., Horikawa, S., and Numa, S., EMBO *2* (1983) 775.
71 Shibasaki, Y., Kawakami, T., Kanazawa, Y., Akanuma, Y., and Takaku, F., J. clin. Invest. *76* (1985) 378.
72 Spiess, J., and Noe, B.D., Proc. natn. Acad. Sci. USA *82* (1985) 277.
73 Steiner, D.F., Terris, S., Chan, S.J., and Rubenstein, A., in: Insulin: Islet Pathology – Islet Function and Insulin Treatment, p. 55. Ed. R. Luft. Lindegren and Soner, Sweden 1976.
74 Steiner, D.F., Quinn, P.S., Chan, S.J., Marsh, J., and Tager, H.S., Ann. N.Y. Acad. Sci. *343* (1980) 1.
75 Steiner, D.F., Kemmler, W., Tager, H.S., Rubenstein, A.H., Lernmark, Å., and Zühlke, H., Proteolytic mechanism in the biosynthesis of polypeptide hormones, in: Proteases and Biological Control, pp. 531–549. Eds E. Reich, D.B. Rifkin, and E. Shaw. Cold Spring Harbor Press 1975.
76 Sun, A.M., Lin, B.J., and Haist, R.E., Can. J. Physiol. Pharmac. *51* (1973) 175.
77 Supattapone, S., Fricker, L.D., and Snyder, S.H., J. Neurochem. *42* (1984) 1017.
78 Talmadge, K., Stahl, S., and Gilbert, W., Proc. natn. Acad. Sci. *77* (1980) 3369.
79 Vlasuk, G.P., Inouye, S., and Inouye, M., J. biol. Chem. *259* (1984) 6195.
80 Vlasuk, G.P., Inouye, S., Ito, H., Itakura, K., and Inouye, M., J. biol. Chem. *258* (1983) 7141.
81 Walter, P., and Blobel, G., Proc. natn. Acad. Sci. USA *77* (1980) 7112.
82 Walter P., and Blobel, G., J. Cell Biol. *91* (1981) 557.
83 Walter P., and Blobel, G., Nature *299* (1982) 691.
84 Walter, P., Biegel, B., Lauffer, L., Garcia, P.O., Ullrich, A., and Harkins, R., in: Protein Transport and Secretion, p. 21. Ed. M.-J. Gething. Cold Spring Harbor Press 1985.
85 Walter, P., Gilmore, R., and Blobel, G., Cell *38* (1984) 5.
86 Wiedman, S.M., Huth, A., and Rapoport, T.A., Nature *309* (1984) 637.
87 Wold, F., A. Rev. Biochem. *50* (1981) 783.
88 Wolfe, P.B., and Wickner, W., Cell *36* (1984) 1067.
89 Zingg, H.H., and Patel, Y.C., Life Sci. *33* (1983) 1241.
90 Zühlke, H., Steiner, D.F., Lernmark, A., and Lipsey, C., CIBA Symp.*41* (1976) 183.
91 Zühlke, H., Kohnert, K.-D., Jahr, H., Schmidt, S., Kirschke, H., and Steiner, D.F., Acta biol. med. germ. *36* (1977) 1695.

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