Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 6834–6838, August 1991
Biochemistry

Isolation and functional expression of a mammalian prohormone processing enzyme, murine prohormone convertase 1

(Kex2/furin/prohormone convertase 3/proopiomelanocortin/*Xenopus* oocytes)

JUDITH KORNER*†, JAY CHUN*†, DAVID HARTER†‡, AND RICHARD AXEL*†‡

*Department of Biochemistry and Molecular Biophysics, ‡Institute of Cancer Research, and †Howard Hughes Medical Institute, Columbia University, New York, NY 10032

Contributed by Richard Axel, May 8, 1991

ABSTRACT **We have combined gene cloning with an assay for prohormone biosynthesis and processing in *Xenopus* oocytes to identify the genes that encode mammalian prohormone processing enzymes. The coinjection of RNA encoding murine prohormone convertase 1 (mPC1), a mammalian endoprotease, along with proopiomelanocortin RNA into an oocyte results in the appropriate cleavage after paired basic residues in the proopiomelanocortin polyprotein necessary to generate corticotropin. The ability of mPC1 to generate corticotropin, along with the observation that mPC1 is specifically expressed in endocrine and neuronal cells, suggests that the mPC1 gene encodes the endopeptidase responsible for the pathway of proopiomelanocortin cleavage observed in the anterior pituitary.**

Many proteins are synthesized as inactive precursors that must be cleaved after paired basic residues to generate biologically active products (1, 2). The requirement for post-translational processing provides a level of control of gene expression distinct from the initial transcriptional and translational events that regulate the synthesis of the inactive precursor molecule. Virtually all mammalian cells for example are capable of cleaving the insulin receptor precursor after paired basic residues in the extracellular domain to yield a biologically active heterodimeric receptor molecule (3). In contrast, gene transfer experiments demonstrate that cleavage at paired basic residues in the insulin precursor occurs only in endocrine cells (4). These observations suggest that animal cells have evolved at least two classes of enzymes specific for cleavage after paired basic residues: a ubiquitous activity present in all cell types and a more tightly regulated cleavage activity designed to meet the individual requirements of specific endocrine and neuronal cell types.

An additional level of regulation is suggested by the observation that different endocrine cells may process the same precursor in different ways. The polyprotein proopiomelanocortin (POMC), for example, encodes at least six peptides. All six peptides, however, are never expressed in the same cell type (refs. 1 and 5; see also Fig. 5*C*). In the cells of the anterior pituitary, a restricted set of cleavages generates corticotropin (ACTH) and β-lipotropin. Additional cleavages are made in the cells of the intermediate lobe to generate a nonoverlapping set of peptides. In this manner, it is possible to generate different combinations of peptides in different cells merely by altering the pattern of processing.

The regulation of protein processing may result from the expression of different endoproteases in different cell types acting on distinct target sites. Alternatively, differential processing may be a consequence of alterations in the structure of either the substrate or the processing enzymes themselves in different subcellular environments. One approach to this problem involves the identification and characterization of the functional properties of the genes encoding these cleavage enzymes. The yeast gene *KEX2* encodes Kex2, a subtilisin-like serine endoprotease (6, 7) that cleaves after paired Lys-Arg residues in the yeast α-mating factor precursor (7). Gene transfer experiments demonstrate that Kex2 can accurately cleave mammalian substrates such as proinsulin (8) and POMC (9, 10). These observations have led to a search for genes homologous to *KEX2* in the mammalian genome. Nucleotide sequencing of the locus encoding the human *fes/fps* protooncogene (11) led to the fortuitous identification of a ubiquitously expressed gene (*fur*) that encodes a protein (furin) that shares significant homology with *KEX2* (12, 13). The isolation of additional homologous genes in the mouse, murine prohormone convertase 1 (mPC1; also called PC3)[§] and 2 (mPC2; refs. 14–16 and this study), suggests that the specificity of prohormone processing results at least in part from the presence of multiple cleavage enzymes. Support for this suggestion, however, requires characterization of the specificity of cleavage by these gene products.

We therefore developed assays for protein processing in *Xenopus* oocytes to study the function of the individual members of this family of endoproteases. We have cloned the gene for the murine endoprotease mPC1, whose expression is restricted to endocrine and neuronal cells. Expression of mPC1 in oocytes results in the specific cleavage of POMC after pairs of basic residues to generate mature ACTH. mPC1 may, therefore, encode the gene responsible for cleavage of POMC in cells of the anterior pituitary.

MATERIALS AND METHODS

Plasmids. Prepro-α-factor cDNA in the plasmid vector pSP65 (Promega) was kindly provided by David Julius (Univ. of Calif., San Francisco). Mouse POMC cDNA (pMKSU16) (17) was subcloned into pBluescript SKII- (Stratagene). *KEX2* cDNA was kindly provided by Robert Fuller (Stanford Univ., Stanford, CA) and Jeremy Thorner (Univ. of Calif., Berkeley) and subsequently subcloned into pGEM-2 (Promega). Mutagenesis of Ser-385 in *KEX2* to Ala was performed with *dut⁻ung⁻ Escherichia coli* using the Bio-Rad Muta-Gene kit (18) generating pKEX-Ser. Recombinant DNA manipulations were performed essentially as described (19).

Microinjection and Metabolic Labeling of *Xenopus* Oocytes. Stratagene or Promega *in vitro* transcription kits were used to synthesize coding-strand RNAs from linearized DNA templates (20). Oocytes were surgically removed from adult *Xenopus laevis* and treated as described (20). Each oocyte

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: mPC1 and mPC2, murine prohormone convertase 1 and 2, respectively; POMC, proopiomelanocortin; ACTH, corticotropin.

[§]The sequence reported in this paper has been deposited in the GenBank data base (accession no. M69196).

was injected with 50 nl of *in vitro*-transcribed RNA at 1 mg/ml per RNA species. Oocytes were metabolically labeled in Barth's medium with Ficoll containing [^{35}S]methionine (2 mCi/ml; >1000 Ci/mmol; 1 Ci = 37 GBq; Amersham) for 23–42 hr. Incubation medium was added to an equal volume of immunoprecipitation buffer (IPB) (21) plus protease inhibitors [0.5 mM leupeptin/0.5 mM phenylmethylsulfonyl fluoride/aprotinin (0.3 μg/ml)]. Oocytes were Dounce-homogenized in 0.8% SDS in water (10 μl per oocyte), boiled for 3 min, and centrifuged at 12,000 × *g* for 15 min. Homogenate supernatants were added to 4 vol of IPB plus inhibitors and stored at −80°C if not immediately immunoprecipitated.

Immunoprecipitation and Gel Electrophoresis. Robert Fuller and Jeremy Thorner kindly provided antiserum against Kex2. Rabbit anti-rat ACTH, synthetic ACTH, and iodinated α-factor were purchased from Peninsula Laboratories. Peninsula also raised rabbit antiserum against a synthetic 13-amino acid α-factor peptide.

Each sample was treated with nonimmune rabbit serum for 30 min at room temperature and then with formalin-fixed *Staphylococcus aureus* cells for 30 min. The mixture was clarified by two 4-min centrifugations in a microcentrifuge at 12,000 × *g* at 4°C. Immune serum was added and incubated with gentle mixing at 4°C for a minimum of 5 hr. *S. aureus* cells were added and rotated for 45 min at room temperature. The immune complexes were pelleted by centrifugation for 30 sec at 4°C and washed (21). The final pellet was resuspended in Laemmli buffer (22) or buffer containing 50 mM Tris·HCl (pH 8.0), 30 mM dithiothreitol, 2% (wt/vol) SDS, 5% 2-mercaptoethanol, and 10 M urea. Samples were heated for 5 min in a boiling water bath and clarified by centrifugation for 5 min at 12,000 × *g*. Immunoprecipitates were normalized to an equivalent number of oocytes per experiment and applied to SDS/polyacrylamide gels (22). Gels were fixed in either 10% (vol/vol) acetic acid/45% (vol/vol) methanol and permeated with Enlightning (NEN/DuPont) or fixed in 10% acetic acid/30% methanol/10% (wt/vol) trichloroacetic acid and subsequently enhanced with 1 M sodium salicylate. Each set of microinjections was repeated at least twice and yielded reproducible results.

Amino-Terminal Mapping of Cleavage Products. Proteins were prepared for sequencing essentially as described (23) and modified (24). Electroblot transfer was carried out for 40–55 min at 411 mA in a Bio-Rad Trans-Blot cell. Blots were exposed to film and bands of interest were excised. Sequencing was carried out on an Applied Biosystems model 477 sequencer using the ATZ program. Fractions were collected, and radioactivity was measured in 10 ml of scintillation fluid.

Cloning of Furin cDNA and Homologs. To isolate a full-length cDNA encoding human furin, oligonucleotide probes based on the 5′ coding sequence of a partial furin clone (11) were used to screen a human hepatoma (HepG2) cDNA library in the vector λZAP (Stratagene). A *Bgl* II–*Xho* I human furin cDNA fragment was then used to probe a cDNA library from AtT-20 poly(A)-selected RNA constructed in the bacteriophage expression vector λZAPII (Stratagene). Plaque hybridization was performed in 10% (vol/vol) formamide/1 M NaCl/10% (wt/vol) dextran sulfate/50 mM Tris·HCl, pH 7.5/1% SDS/denatured salmon sperm DNA (0.1 mg/ml) at 42°C. Filters were washed in hybridization solution without dextran sulfate at 42°C. Sequences of excised plasmids were obtained (25) and assembled using the IBI MacVector 3.0 (26) program.

Northern Blot Analysis. Rat pituitary, mouse striatum, RIN (rat insulinoma), and WEHI-3 (mouse monocytes) poly(A) RNA were generous gifts from Stuart Sealfon and Jim Roberts (Mount Sinai, New York), Ellen Robey (Columbia University, New York), Andrew Murphy and Argiris Efstratiadis (Columbia University), and Silvana Obici (Columbia University), respectively. HIT (hamster insulinoma) cells were described by Robert Santerre *et al.* (27). AtT-20 cells were obtained from the American Type Culture Collection. Human γ-actin probe (28) was used as a control. RNA was fractionated on 1% agarose/formaldehyde gels and transferred to GeneScreen (NEN/DuPont) (19).

RESULTS

Prohormone Processing in *Xenopus* Oocytes. In initial experiments, we used the genes encoding the α-factor precursor and the yeast endopeptidase Kex2 to develop a model system to examine proteolytic processing in *Xenopus* oocytes. The α-factor prohormone is normally cleaved in yeast by Kex2 at the carboxyl side of four pairs of basic amino acids to generate four intermediates, each containing an α-factor sequence (Fig. 1*B*; refs. 6 and 29). To determine whether processing of pro-α-factor also occurs in *Xenopus* oocytes, we injected oocytes with *in vitro*-synthesized RNA encoding

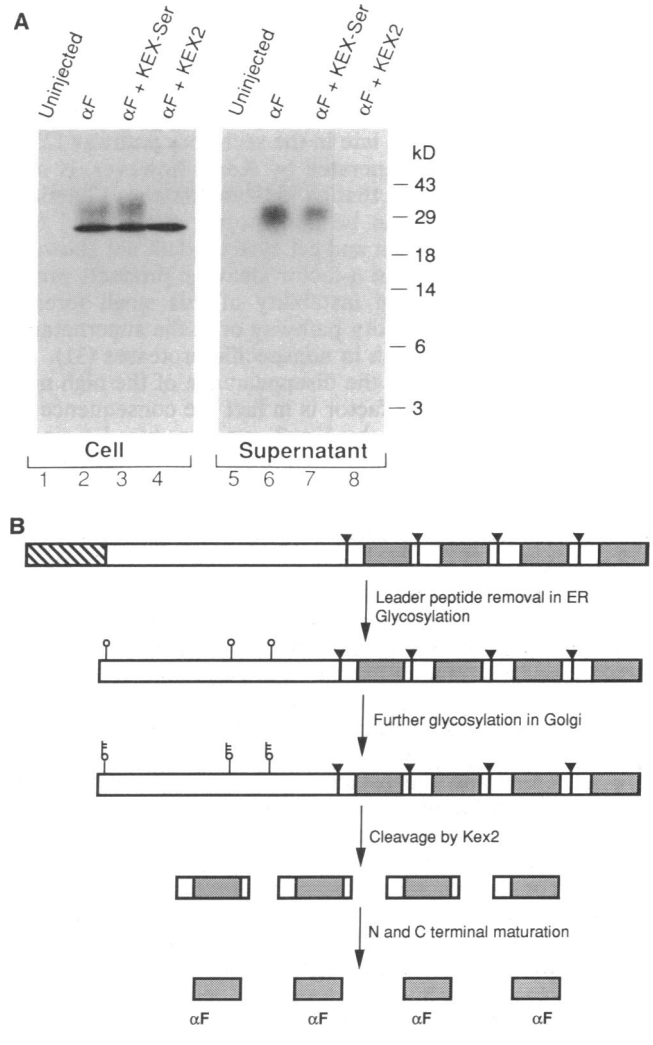

FIG. 1. Cleavage of the α-mating factor precursor in *Xenopus* oocytes. (*A*) Oocytes were microinjected with *in vitro*-synthesized RNA encoding prepro-α-factor (αF), along with either KEX2 RNA or RNA encoding KEX2 mutated at the active-site serine, KEX-Ser. Oocyte lysates (lanes 1–4) and incubation medium (lanes 5–8) were immunoprecipitated with polyclonal antiserum directed against the mature α-factor peptide and electrophoresed on an 18% polyacrylamide gel containing SDS. (*B*) Post-translational processing of prepro-α-factor in yeast (29). The α-factor precursor encodes four copies of the 13-amino acid hormone. The signal sequence (hatched box) and three sites for addition of asparagine-linked carbohydrate (open circles) are shown (30). Sites of cleavage at Lys-Arg pairs by the KEX2 endoprotease are indicated by triangles.

the α-factor precursor. Protein products were analyzed by immunoprecipitation with antiserum directed against mature α-factor. As shown in Fig. 1*A*, lane 2, two unprocessed forms of the α-factor precursor are present in oocyte extracts: a discrete 26-kDa immunoreactive product and a broader band between 26 and 32 kDa. These products are larger than the 18.6-kDa *in vitro*-translated product of α-factor RNA (data not shown) but correspond to the α-factor precursors observed in yeast. The 26-kDa product is likely to result from N-linked glycosylation within the endoplasmic reticulum and the higher molecular mass products (26–32 kDa) probably result from additional polymerization of sugars as the precursor progresses through the secretory pathway (Fig. 1*B*; refs. 21 and 29). The observation that only the diffuse high molecular mass forms appear in the supernatant is consistent with this interpretation (Fig. 1*A*, lane 6).

Coinjection of RNA encoding the Kex2 endoprotease along with the α-factor precursor RNA results in the complete disappearance of the α-factor precursor from the oocyte supernatant and in the selective disappearance of the higher molecular mass forms of α-factor from the oocyte extract (Fig. 1*A*). The selective loss of the higher molecular mass forms of the α-factor precursor suggests that, as in yeast, cleavage by Kex2 occurs late in the secretory pathway (21). The cleavage product generated by Kex2, however, is not observed despite the fact that an iodinated mature 13-amino acid α-factor peptide can be immunoprecipitated and detected with this antiserum and gel system (data not shown). The inability to detect the α-factor cleavage products probably reflects an inherent instability of this small foreign peptide within the secretory pathway or in the supernatant, which is known to be rich in nonspecific proteases (31).

To determine whether the disappearance of the high molecular mass forms of α-factor is in fact the consequence of endoproteolytic cleavage by Kex2, we introduced a single amino acid substitution into Kex2, replacing the active site serine with alanine. This mutant, KEX-Ser, is detected in oocytes at levels higher than the wild-type enzyme (Fig. 2) but has no detectable proteolytic activity on fluorogenic substrates *in vitro* (data not shown). If Kex2 is responsible for the degradation of the α-factor precursor by a mechanism other than endoprotease activity, we should also observe degradation with KEX-Ser. On the other hand, if the disappearance of the α-factor precursor is a consequence of Kex2 proteolytic action, we would predict that the high molecular mass forms would not be degraded in the presence of KEX-Ser. As shown in Fig. 1*A*, coinjection of RNAs encoding KEX-Ser and the α-factor prohormone results in the synthesis of α-factor precursors identical to that observed upon injection of the α-factor precursor RNA alone. These data suggest that the oocyte is incapable of appropriate cleavage of the α-factor precursor but that this precursor can indeed be processed if exogenous Kex2 is expressed in the oocyte. In similar experiments using *Xenopus* oocytes, we have shown that the *Aplysia* egg-laying hormone is processed from the egg-laying hormone precursor only upon coinjection of Kex2 RNA (J.K., J.C., and R.A., unpublished data). Thus these results indicate that *Xenopus* oocytes may provide a facile system to examine the function of the genes encoding mammalian endoproteases.

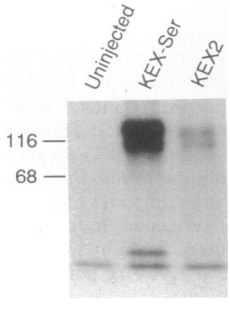

FIG. 2. Immunoprecipitation of KEX2 and KEX-Ser from oocyte extracts. Oocytes injected with equal amounts of RNA encoding either Kex2 or the mutant KEX-Ser were metabolically labeled and immunoprecipitated with antisera against the wild-type enzyme. Samples were analyzed by SDS/PAGE (8% gels). Identical results were obtained in three experiments. Molecular masses in kDa are indicated.

Isolation and Characterization of a Tissue-Specific Mammalian Endopeptidase, mPC1. A full-length human furin cDNA was used as a hybridization probe to identify homologous genes expressed in the anterior pituitary cell line AtT-20. A cDNA clone containing a 2.5-kilobase (kb) insert that encodes a 753-amino acid open reading frame was isolated. This sequence is identical to the cDNA clones mPC1 (15) and PC3 (16). The deduced amino acid sequence of mPC1 exhibits 46% identity with both furin and mPC2, 33% identity with Kex2, and 25% identity with subtilisin (Fig. 3). Significantly greater homology is observed in the regions surrounding the putative active sites (Asp-167, His-208, and Ser-382) that form the catalytic triad characteristic of the subtilisin family of serine proteases (34). In addition, Asn-309, which is thought to stabilize the transition complex (35), is conserved in mPC1, furin, Kex2, and subtilisin. Within these regions, a number of residues are conserved in the eukaryotic proteins but differ from the corresponding residues in subtilisin. For example, the alanine residue immediately after the active site serine of each eukaryotic protein (Ser-382 in mPC1) replaces methionine, which is conserved in all known *Bacillus* subtilisins (35). This change may be of functional significance since rapid inactivation of subtilisin is coincidental with oxidation of this methionine residue *in vitro* (36). It is of interest that the subtilisins must be cleaved, perhaps autocatalytically, to generate an active enzyme (37, 38). The presence of clusters of basic amino acids within the amino terminus of the eukaryotic proteins may constitute cleavage sites for removal of a prosequence.

Northern blot analysis indicates that, unlike furin, which is expressed in all tissues (39), mPC1 expression is restricted to cells and tissues of neuroendocrine origin (Fig. 4). A 2.7-kb RNA is expressed in the pituitary and in the AtT-20 cell line. A minor 5.5-kb transcript is also observed in pituitary RNA and in RNA from AtT-20 cells and two insulinoma cell lines. The insulinoma cell lines exhibit additional hybridizing bands, but the coding potential of these RNAs has not been examined. Expression is not detectable in heart, lung, muscle, and spleen (data not shown).

mPC1 Cleaves POMC. The expression of mPC1 in anterior pituitary cells suggests that this gene may encode the enzyme responsible for the specific cleavage of the POMC precursor to generate mature ACTH (Fig. 5*C*). POMC RNA was, therefore, injected into *Xenopus* oocytes and the protein products were then immunoprecipitated with antisera directed against mature ACTH. Injection of POMC RNA results in the synthesis of two proteins of between 30 and 35 kDa (Fig. 5*A*) that correspond to the POMC precursors observed *in vivo* (40). Mature ACTH is not detected.

However, when oocytes are coinjected with RNA transcripts encoding POMC and either mPC1 or Kex2, an immunoreactive cleavage product is generated with a molecular mass of ≈7 kDa (Fig. 5*A*). This product comigrates with a synthetic ACTH peptide that also appears as a broad band under our gel conditions (data not shown). Radioactive sequencing of this 7-kDa peptide reveals a methionine residue at the fourth position from the amino terminus (Fig. 5*B*). This sequence corresponds to cleavage of POMC after the Lys-Arg pair (positions 122 and 123) immediately preceding ACTH (Fig. 5*C*). These data indicate that the mPC1 cDNA encodes an endoprotease that cleaves after pairs of basic residues in POMC to generate mature ACTH.

DISCUSSION

Most neuroactive peptide hormones are synthesized and cleaved from a large precursor molecule that often contains several peptide sequences (1, 2). Gene cloning has identified

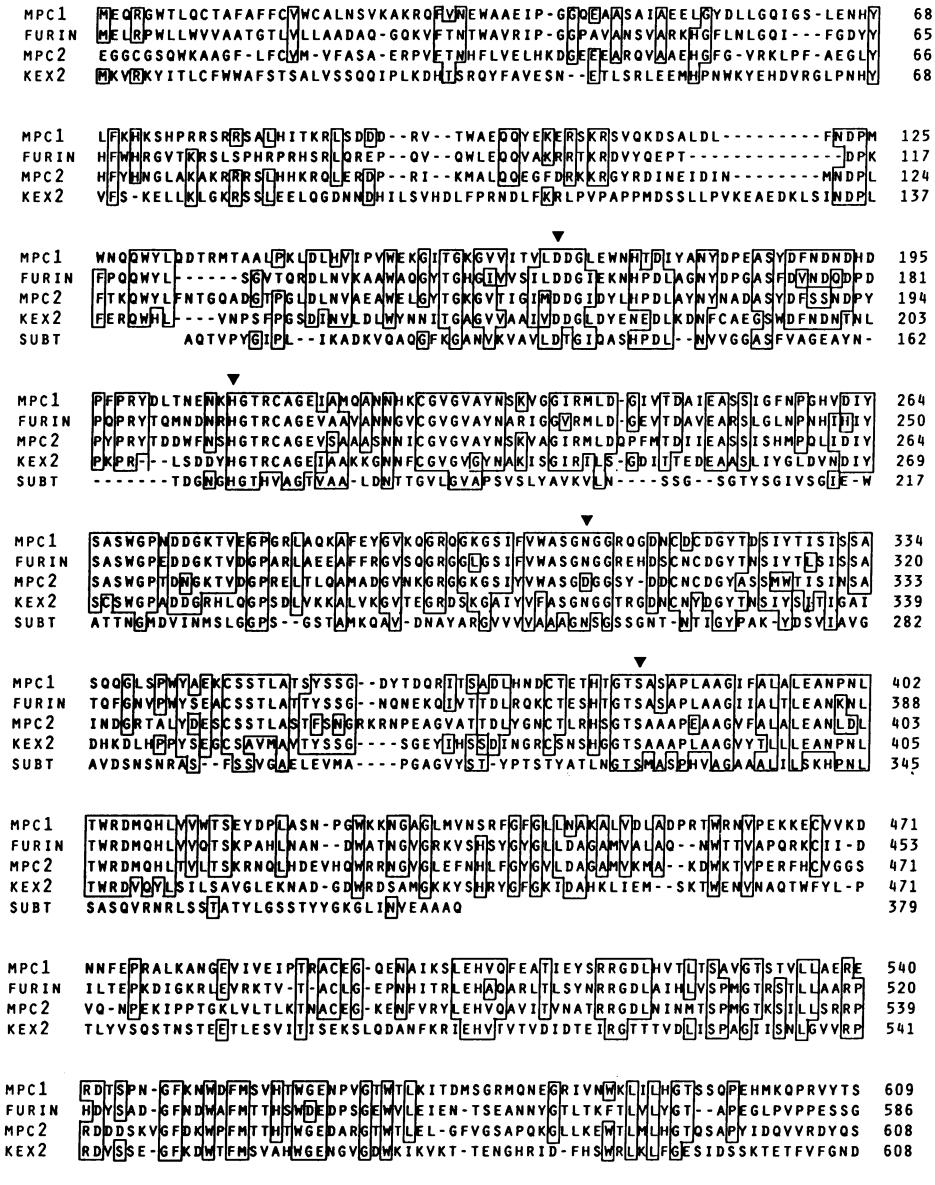

FIG. 3. Alignment of the deduced amino acid sequences of mPC1, furin (32), mPC2 (14), KEX2 (7), and subtilisin [SUBT, *Bacillus licheniformis* subtilisin Carlsberg (33)]. Boxed amino acids are identical between at least three of the proteins. The active site residues are indicated by triangles. Gaps (dashes) were introduced for maximum alignment.

a family of mammalian genes homologous to the subtilisin endoproteases. The product of one member of this family, the furin gene, is ubiquitously expressed and is capable of processing nerve growth factor (41) and von Willebrand factor (13) precursor proteins. Additional genes have been identified (e.g., mPC1 and mPC2) whose expression is restricted to endocrine and neuronal cells (14–16). These data suggest that the specificity of the proteolytic processing of protein precursors may result from the regulated expression of different endopeptidases in different cell types.

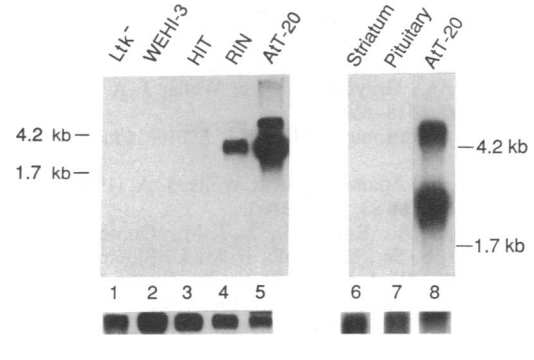

FIG. 4. Northern blot analysis of mPC1 expression. Polyadenylylated RNAs (0.7–2 μg) from cells and tissues as indicated were electrophoresed on 1% agarose/formaldehyde gels. (*Upper*) Probed for mPC1. (*Lower*) Same blots reprobed for human γ-actin.

In this study, we have combined gene cloning with an assay for prohormone biosynthesis and processing in *Xenopus* oocytes to identify the genes responsible for the cleavage of the POMC polyprotein. The coinjection of mPC1 and POMC RNAs into an oocyte results in the appropriate cleavage after paired basic residues in the POMC precursor necessary to generate ACTH. The ability of mPC1 to generate ACTH, along with the observation that mPC1 is specifically expressed in endocrine and neuronal cells, suggests that the mPC1 gene encodes the endopeptidase responsible for the pathway of POMC cleavage observed in the anterior pituitary. In the intermediate lobe of the pituitary, ACTH undergoes additional internal cleavage, suggesting the expression of a distinct endopeptidase activity (Fig. 5*C*; ref. 1). Interestingly, *in situ* hybridization reveals mPC1 expression in the anterior and intermediate lobes of the pituitary, whereas mPC2 expression is restricted to the intermediate lobe (15). This selective expression makes mPC2 a likely candidate gene encoding the endopeptidase that cleaves ACTH to α-melanotropin and corticotropin-like intermediate lobe peptide. Thus, alternative processing of the POMC polyprotein may be the consequence of the regulated expres-

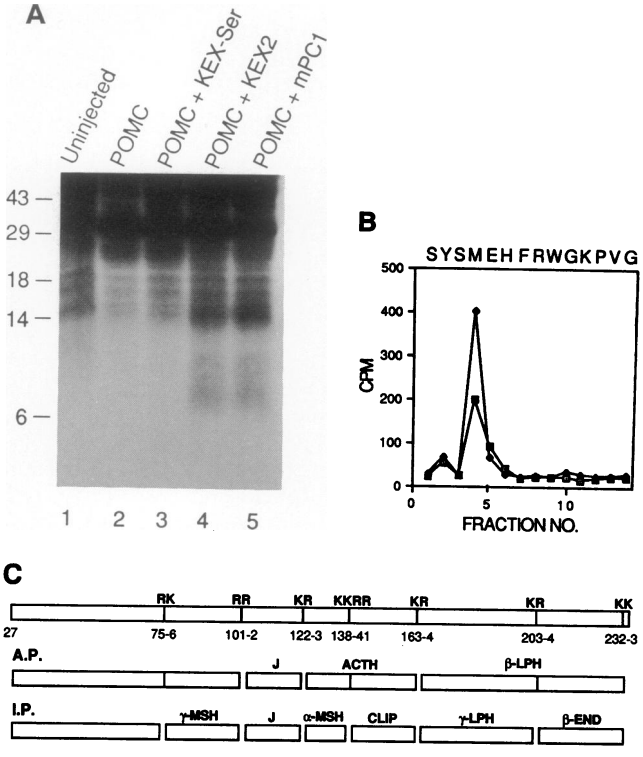

FIG. 5. mPC1 cleaves POMC to generate mature ACTH. (*A*) Oocytes were injected with *in vitro*-synthesized RNA encoding mouse POMC, along with RNA encoding KEX-Ser, KEX2, or mPC1. Antiserum directed against the mature ACTH peptide was used to immunoprecipitate [^{35}S]methionine-labeled cell lysates. The products were analyzed by SDS/PAGE (18% gel). Molecular masses in kDa are indicated. (*B*) The 7-kDa cleavage products resulting from the coexpression of POMC and KEX2 (□) or POMC and mPC1 (◆) were analyzed by radiolabeled amino-terminal mapping. Each product yields a peak of radioactivity ([^{35}S]methionine cpm) at the fourth position from the amino terminus. The predicted amino-terminal sequence of ACTH is shown (17). (*C*) Schematic representation of the mouse POMC polyprotein and the predominant cleavage products generated in the anterior (A.P.) and intermediate (I.P.) lobes of the pituitary. A fraction of β-lipotropin (β-LPH) is also cleaved in the anterior lobe (5, 17). The name (single-letter code) and positions of amino acids at cleavage sites are shown. MSH, melanotropin; CLIP, corticotropin-like intermediate lobe peptide; END, endorphin; J, joining peptide.

sion of endopeptidases with subtly different specificities in distinct pituitary cell types.

We acknowledge Mary Ann Gawinowicz for her expertise in peptide sequence analysis; Laura O'Bryan for help with various aspects of this project, especially with the site-directed mutagenesis; Yonghua Sun and Adriana Nemes for their technical assistance; and Andrew Furley for his help with sequence analysis. We are grateful to Robert Fuller and Jeremy Thorner for the gift of Kex2 antiserum. We thank Thomas Jessell for his helpful suggestions. We are also grateful to Phyllis Jane Kisloff for her help in the preparation of this manuscript. This work was supported by a predoctoral fellowship from Columbia University (J.K.), The Howard Hughes Medical Institute (R.A.), and by a grant from the National Cancer Institute POICA23767 (R.A., D.H., and J.C.).

1. Douglass, J., Civelli, O. & Herbert, E. (1984) *Annu. Rev. Biochem.* **53**, 665–715.
2. Sossin, W. S., Fisher, J. M. & Scheller, R. H. (1989) *Neuron* **2**, 1407–1417.
3. Rosen, O. M. (1987) *Science* **237**, 1452–1458.
4. Moore, H.-P. H., Walker, M. D., Lee, F. & Kelly, R. B. (1983) *Cell* **35**, 531–538.
5. Schnabel, E., Mains, R. E. & Farquhar, M. G. (1989) *Mol. Endocrinol.* **89**, 1223–1235.
6. Julius, D., Brake, A., Blair, L., Kunisawa, R. & Thorner, J. (1984) *Cell* **37**, 1075–1089.
7. Mizuno, K., Nakamura, T., Ohshima, T., Tanaka, S. & Matsuo, H. (1988) *Biochem. Biophys. Res. Commun.* **156**, 246–254.
8. Thim, L., Hansen, M. T., Norris, K., Hoegh, I., Boel, E., Forstrom, J., Ammerer, G. & Fiil, N. P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6766–6770.
9. Thomas, G., Thorne, B. A., Thomas, L., Allen, R. G., Hruby, D. E., Fuller, R. & Thorner, J. (1988) *Science* **241**, 226–230.
10. Germain, D., Zollinger, L., Racine, C., Gossard, F., Dignard, D., Thomas, D. Y., Crine, P. & Boileau, G. (1990) *Mol. Endocrinol.* **4**, 1572–1579.
11. Roebroek, A. J. M., Schalken, J. A., Leunissen, A. M., Onnekink, C., Bloemer, H. P. J. & Van de Ven, W. J. M. (1986) *EMBO J.* **5**, 2197–2202.
12. Fuller, R. S., Brake, A. J. & Thorner, J. (1989) *Science* **246**, 482–486.
13. Wise, R. J., Barr, P. J., Wong, P. A., Kiefer, M. C., Brake, A. J. & Kaufman, R. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9378–9382.
14. Seidah, N. G., Gaspar, L., Mion, P., Marcinkiewicz, M., Mbikay, M. & Chrétien, M. (1990) *DNA Cell Biol.* **9**, 415–424.
15. Seidah, N. G., Marcinkiewicz, M., Benjannet, S., Gaspar, L., Beaubien, G., Mattei, M. G., Lazure, C., Mbikay, M. & Chrétien, M. (1991) *Mol. Endocrinol.* **91**, 111–122.
16. Smeekens, S. P., Avruch, A. S., LaMendola, J., Chan, S. J. & Steiner, D. F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 340–344.
17. Uhler, M. & Herbert, E. (1983) *J. Biol. Chem.* **258**, 257–261.
18. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 488–492.
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
20. Nakanishi, N., Shneider, N. A. & Axel, R. (1990) *Neuron* **5**, 569–581.
21. Julius, D., Schekman, R. & Thorner, J. (1984) *Cell* **36**, 309–318.
22. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
23. Matsudaira, P. (1987) *J. Biol. Chem.* **262**, 10035–10038.
24. Kennedy, T. E., Gawinowicz, M. A., Barzilai, A., Kandel, E. R. & Sweatt, J. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7008–7012.
25. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
26. Pustell, J. M. (1988) *Nucleic Acids Res.* **16**, 1813–1820.
27. Santerre, R. F., Cook, R. A., Crisel, R. M. D., Sharp, J. D., Schmidt, R. J., Williams, D. C. & Wilson, C. P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4339–4343.
28. Enoch, T., Zinn, K. & Maniatis, T. (1986) *J. Mol. Cell. Biol.* **6**, 801–810.
29. Fuller, R. S., Sterne, R. E. & Thorner, J. (1988) *Annu. Rev. Physiol.* **50**, 345–362.
30. Kurjan, J. & Herskowitz, I. (1982) *Cell* **30**, 933–943.
31. Soreq, H. & Miskin, R. (1981) *FEBS Lett.* **128**, 305–310.
32. van den Ouweland, A. M. W., van Duijnhoven, H. L. P., Keizer, G. D., Dorssers, L. C. J. & Van de Ven, W. J. M. (1990) *Nucleic Acids Res.* **18**, 664.
33. Jacobs, R., Eliasson, M., Uhlen, M. & Flock, J. I. (1985) *Nucleic Acids Res.* **13**, 8913–8926.
34. Kraut, J. (1977) *Annu. Rev. Biochem.* **46**, 331–358.
35. Wells, J. A. & Estell, D. A. (1988) *Trends Biochem. Sci.* **13**, 291–297.
36. Estell, D. A., Graycar, T. P. & Wells, J. A. (1985) *J. Biol. Chem.* **260**, 6518–6521.
37. Ikemura, H. & Inouye, M. (1988) *J. Biol. Chem.* **263**, 12959–12963.
38. Power, S. D., Adams, R. M. & Wells, J. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3096–3100.
39. Schalken, J. A., Roebroek, A. J. M., Oomen, P. P. C. A., Wagenaar, S. Sc., Debruyne, F. M. J., Bloemers, H. P. J. & Van de Ven, W. J. M. (1987) *J. Clin. Invest.* **80**, 1545–1549.
40. Eipper, B. A. & Mains, R. E. (1980) *Endocr. Rev.* **1**, 1–26.
41. Bresnahan, P. A., Leduc, R., Thomas, L., Thorner, J., Gibson, H. L., Brake, A. J., Barr, P. J. & Thomas, G. (1990) *J. Cell Biol.* **111**, 2851–2859.