Endocrine Journal 1998, **45** (4), 569–573

NOTE

Characterization of the Promoter of the Mouse Prohormone Convertase PC2 Gene

SHINYA OHAGI, HITOMI TATSUTA, TOKIO SANKE, HIROTO FURUTA, MACHI FURUTA, TADASHI HANABUSA, AND KISHIO NANJO

The First Department of Medicine, Wakayama University of Medical Science, Wakayama 640, Japan

Abstract. Prohormone convertase 2 (PC2) is a member of a family of mammalian subtilisin-like endoproteases that are involved in the processing of prohormones, neuropeptides and many other precursor derived proteins. The expression of PC2 is restricted to neuroendocrine tissues such as pancreatic islets, the pituitary and the brain. To understand the regulation of the PC2 gene, we cloned and characterized the promoter region of the mouse PC2 gene. The transcriptional start site of the mouse PC2 gene is identical to that of the human. There is 79% identity in the sequences of the promoter regions between the mouse and human PC2 genes. The mouse PC2 gene, like the human, does not have a TATA-like motif in the region just upstream of the start of the transcription. Studies with promoter-reporter gene, chrolamphenicol acetyltransferase (CAT), constructs showed that the region from −400 to −170 bp was necessary for high level expression of the mouse PC2 gene in the βTC-3 insulinoma cells.

Key words: PC2, Mouse, Promoter, CAT assay

(*Endocrine Journal* **45**: 569–573, 1998)

LIMITED proteolysis at pairs of basic amino acids is characteristic of a family of subtilisin-like endoproteases related to the yeast Kex2 gene product [1, 2]. Several mammalian homologues of Kex2 have been recently identified [3, 4]. One of these, furin appears to be responsible for proprotein processing in the constitutive secretory pathway [5]. In contrast, prohormone convertase 2 (PC2) and prohormone convertase 3 (PC3) are involved in the processing of prohormones such as proinsulin and proopiomelanocortin which are secreted through the regulated pathway [6]. Furin is expressed in a wide variety of tissues and cells with high levels of expression in the liver and the kidneys, whereas PC2 and PC3 are more restrictedly distributed, primarily in tissues that synthesize and secrete peptide hormones including the pituitary and endocrine pancreas. In order to obtain more understanding of the factors regulating the expression of the PC2 gene, we have isolated and characterized the promoter region of the mouse PC2 gene.

Received: October 27, 1997
Accepted: February 20, 1998
Correspondence to: Dr. Kishio NANJO, The First Department of Medicine, Wakayama University of Medical Science, 27 Nanaban-cho, Wakayama 640, Japan

Materials and Methods

Isolation of the promoter region of the mouse PC2 gene

Mouse genomic DNA library (Clontech, Palo Alto, CA) was screened by hybridization with a fragment of the mouse PC2 cDNA predicted to represent exon 1 based on the exon-intron organization of the human PC2 gene [7]. The sequence of the promoter region was determined after cloning appropriate fragments into pGEM-3Z (Promega, Madison, WI) with Sequenase Version 2.0 (United States Biochemical, Cleveland, OH) with

either universal or specific primers.

Identification of the transcriptional start site

The transcriptional start site was localized by primer extension analysis. The oligonucleotide (5'-CAAATCTGTACGCTGTCCAC-3') complementary to nucleotides −173 to −192 relative to the translation start site was labeled with [r-32P]ATP using T4 polynucleotide kinase, annealed with 20 μg of the βTC-3 total RNA, and extended with avian myeloblastosis virus reverse transcriptase. The primer extension products were separated by electrophoresis in a DNA sequencing gel, and a sequencing ladder obtained with the same primer on the appropriate template was run in adjacent lanes to allow the start site to be previously localized.

Localization of cis-acting regulatory sequences in the mouse PC2 gene

A 1.8 Kb EcoRI-HindIII fragment containing 1.6 kb of 5'-flanking region and 172 bp of the 5'-untranslated region of the mouse PC2 gene was isolated and cloned into a chrolamphenicol acetyltransferase (CAT) expression reporter vector, pSV00CAT [8]. A nested series of different deletion mutants were prepared with PCR and also ligated into pSV00CAT. The sequence of the inserts in each construct was confirmed by DNA sequencing. The activity of these constructs was measured in mouse insulinoma βTC-3 cells and human hepatoma HepG2 cells. For transfection studies, cells were trypsinized, seeded at 1×10^6 cells/100 mm tissue culture dish and incubated overnight. The medium was removed and the cells were washed twice with Opti-MEM I medium (GIBCO/BRL). Ten μg of each construct together with 50 μl of lipofectin (GIBCO/BRL) was used. Each transfection also included 2 μg of pSV-β-Galactosidase (Promega) as a control for transfection efficiency. After 24 h, the cells were harvested, and CAT and β-galactosidase were assayed. The CAT activity was corrected with respect to that of β-galactosidase.

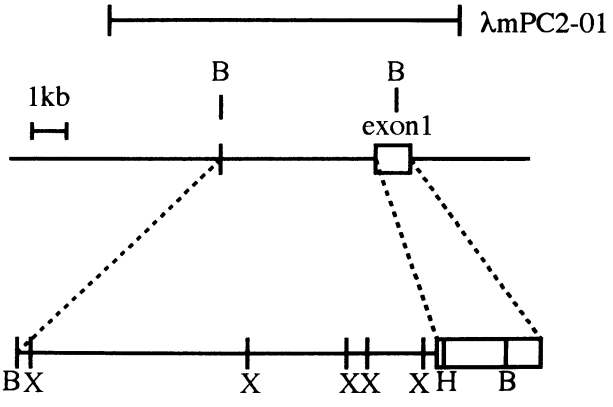

Fig. 1. Structure of the promoter region of the mouse PC2 gene. A schematic representation of a mouse PC2 recombinant, λmPC2-01, was shown at the top. An extended map of the promoter and exon 1 region was at the bottom. Abbreviations for restriction enzymes are: B, BamHI; H, HindIII; X, XbaI.

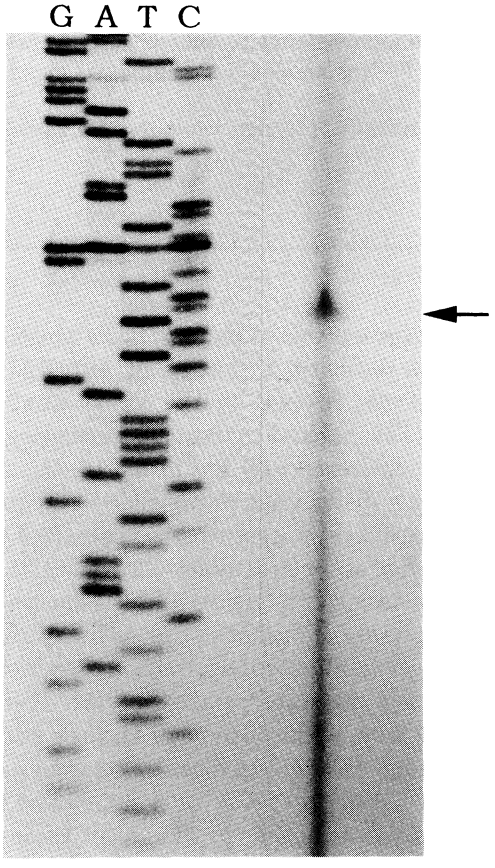

Fig. 2. Localization of the transcriptional start site in the mouse PC2 gene by primer extension. The primer extended product is noted by the arrow. The sequence ladder shown on the left was obtained using the same primer as used for primer extension with the appropriate template.

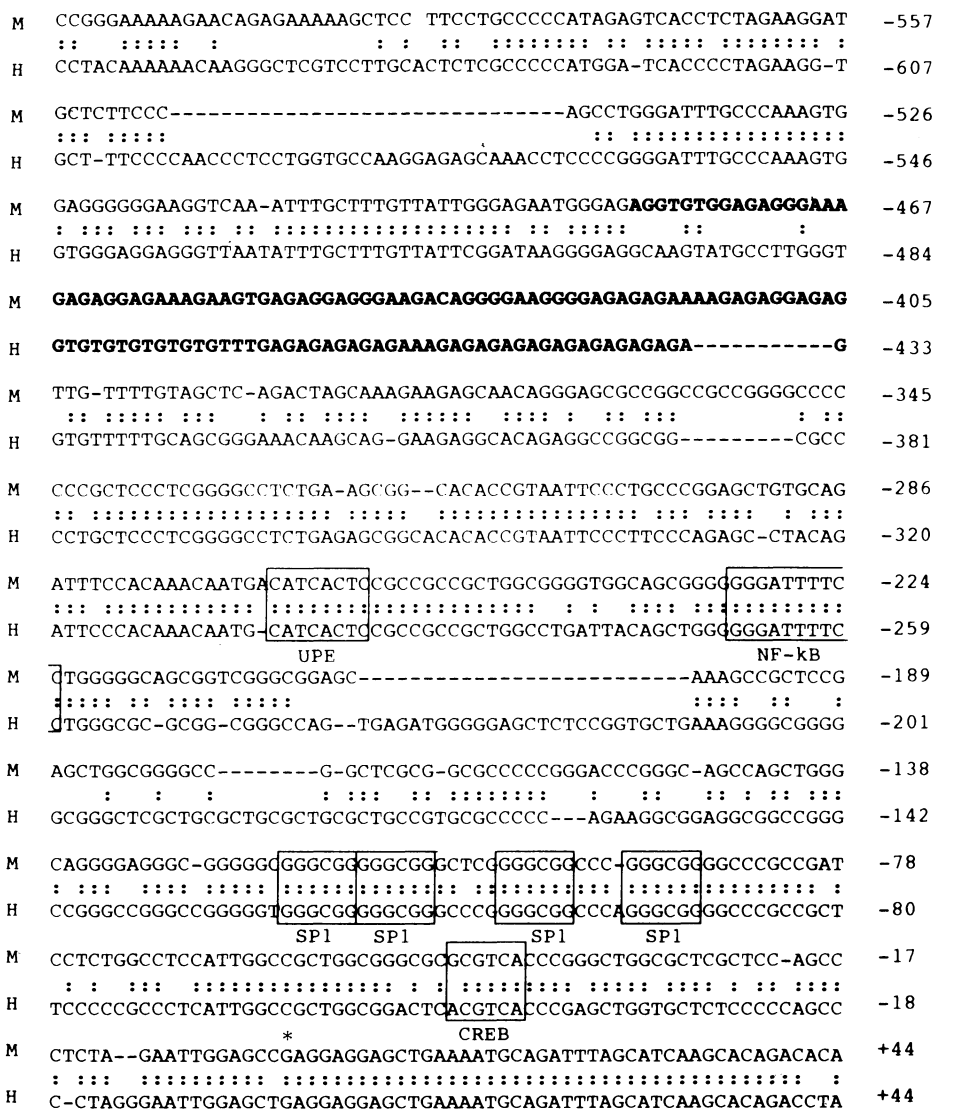

Fig. 3. Sequence of the promoter region of the mouse PC2 gene and comparison with corresponding region of the human PC2 gene. The M and H denote mouse and human sequences, respectively. Numbers on the right side of the sequences indicate nucleotide positions in relation to the transcription initiation site (*). Gaps in the alignment are indicated by dashes. Column means identical nucleotides. Potential binding sites for transcriptional factor SP1 and consensus sequences for the putative regulatory elements (UPE, NF-kB, and CRE) are boxed. The purine-rich regions in the mouse and human genes are shown in bold letters.

Results and Discussion

The promoter region of the mouse PC2 gene was isolated by hybridization with the mPC2 cDNA probe corresponding to exon1 of the human PC2 gene [7]. The partial sequence of the insert in λmPC2 -01 which includes 6 kb of the promoter region and 310 bp of the 5'-untranslated region is shown in Fig. 1. Primer extension studies localized the transcriptional start site to nucleotide −332 bp relative to the start of the translation (Fig. 2). Transcription of the mouse PC2 gene proved to begin at the corresponding location in human PC2 sequence. The mouse PC2 promoter sequence, like the human, was very GC-rich, showing no evidence of TATA [9] or CAAT [10] motifs. Alignment of the mouse and human [7] promoter sequences showed extension regions of homology extending for more than 500 bp upstream of the start of

trancription (Fig. 3). The overall identity between the two promoters was 79%.

A number of putative transcription regulatory elements were conserved between the mouse and the human implying that they are functionally important. These included four putative SP1 binding sites [11], a cAMP response element (CRE) [12], binding sites for nuclear factor kappa B (NF-kB) [13] and on upstream promoter element (UPE) [14]. The UPE is a motif found in the glucokinase gene which is also expressed in insulin-secreting cells as the PC2 gene. There is also a purine-rich region [15] present in the promoter region of the mouse gene although there are differences between the mouse and human genes in the size and the sequence organization of this region.

A genomic fragment from −1.8 kb to +170 bp was cloned upstream of a CAT reporter vector, pSVOOCAT. This construct was active in the βTC-3 cells (Fig. 4) which express PC2 mRNA. The activity of this construct in HepG2 cell which do not express PC2 mRNA was as low as 11.5%–20% of that observed in βTC-3 cells. Analyses of a series of nested deletions showed that the region from −400 to −170 bp contained sequences required for high level expression of the mouse PC2 gene in βTC-3 cells.

In conclusion, we have characterized the promoter region of the mouse PC2 gene and shown that this sequence shares regions of identity with the human PC2 promoter described previously [7]. These conserved regions may be of functional importance in the control of PC2 gene expression. From the result of the CAT assay, the region from −400 to −170 was seen to be indispensable for the expression of the mouse PC2 gene. Further studies are required to identify the factors that regulate the expression of the PC2 gene.

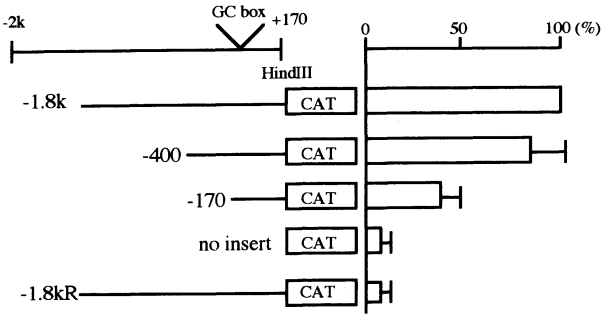

Fig. 4. Structure of CAT reporter gene constructs and their relative CAT activities in the β-TC3 cells. The relative CAT activity is measured as the ratio of the value of each construct to that of the largest construct (−1.8 kb to +170 bp) and expressed as the mean (+SD) percentage. The results presented are the average from at least three independent experiments performed in duplicate. Error bars show S.D. −1.8KR; −1.8K in the reverse orientation.

Acknowledgements

We thank Dr. Graeme I. Bell at the University of Chicago for advice and helpful suggestions. This work was supported in part by a Grant in Aid for Creative Basic Research (10NP0201) from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Docherty K, Steiner DF (1982) Post-translational proteolysis in polypeptide hormone biosynthesis. *Annu Rev Physiol* 44: 625–638.
2. Fuller RS, Brake AJ, Thorner J (1989) Intracellular targeting and structural conservation of a prohormone-processing endoprotease. *Science* 246: 482–486.
3. Steiner DF, Smeekens SP, Ohagi S, Shan SJ (1992) The new enzymology of precursor processing endopeptidases. *J Biol Chem* 267: 23435–23438.
4. Seidah NG, Chretien M (1992) Proprotein and prohormone convertase of the subtilisin family. Recent development and future perspectives. *Trends Endocrinol Metab* 3: 113–140.
5. Roebroek AJM, Schalken JA, Leunissen JAM, Onnekink C, Bloemers HPJ, Van de Ven, WJM (1986) Evolutionary conserved close linkage of the c-fes/fps proto-oncogene and genetic sequences encoding a receptor-like protein. *EMBO J* 5: 2197–2202.
6. Smeekens SP, Montag AG, Thomas G, Albiges-Rizo C, Carroll R, Bening M, Phillips LA, Martin S, Ohagi S, Gardner P, Swift HH, Steiner DF (1992) Proinsulin processing by the subtilisin-related proprotein convertases furin, PC2, and PC3. *Proc Natl Acad Sci USA* 89: 8822–8826.

7. Ohagi S, LaMendola J, LeBeau MM, Espinosa R III, Takeda J, Smeekens SP, Chan SJ, Steiner DF (1992) Identification and analysis of the gene encoding human PC2 gene, a prohormone convertase expressed in neuroendocrine tissues. *Proc Natl Acad Sci USA* 89: 4977–4981.
8. Gorman CM, Moffat LF, Howard BH (1982) Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol Cell Biol* 2: 1044–1051.
9. Breathnach R, Chambon L (1981) Organization and expression of eukaryotic split genes coding for proteins. *Ann Rev Biochem* 50: 349–383.
10. Chodosh LA, Badwin AS, Carthew RW, Sharp PA (1988) Human CCAAT-binding proteins have heterogeous subunits. *Cell* 53: 11–24.
11. Briggs MR, Kadonaga JT, Bell SP, Tjian R (1986) Purification and biochemical characterization of the promoter-specific transcription factor SP1. *Science* 234: 47–52.
12. Roester WJ and Vandenbark GR, Hanson RW (1988) Cyclic AMP and the induction of eukaryotic gene transcription. *J Biol Chem* 263: 9063–9066.
13. Chu R, Imagawa M, Imbra RJ, Bockoven JR, Karin M (1987) Multiple cis- and trans-acting elements mediate the transcriptional response to phorbol esters. *Nature* 332: 557–561.
14. Magnuson MA, Jetton TL (1993) Evolutionary conservation of elements in the upstream glucokinase promoter. *Bioche Soc Trans* 21: 160–163.
15. Berg DT, Walla JD, Reifel-Miller AE, Grinnell BW (1989) E1A-induced enhancer activity of the poly (dG-dT) poly (dA-dC) element (GT element) and interactions with a GT-specific nuclear factor. *Mol Cell Biol* 9: 5248–5253.