Identification and analysis of the gene encoding human PC2, a prohormone convertase expressed in neuroendocrine tissues

(peptide hormone/precursor processing/subtilisin-like protease/chromosome 20/promoter)

SHINYA OHAGI*, JOSEPH LAMENDOLA*†, MICHELLE M. LEBEAU‡, RAFAEL ESPINOSA III‡, JUN TAKEDA*†, STEVEN P. SMEEKENS*†, SHU JIN CHAN*†, AND DONALD F. STEINER*†§

†The Howard Hughes Medical Institute, Departments of *Biochemistry and Molecular Biology, and ‡Medicine, The University of Chicago, Chicago, IL 60637

Contributed by Donald F. Steiner, February 21, 1992

ABSTRACT In recent studies we have identified PC2 and PC3, members of a family of serine proteases that are related structurally to subtilisin, and have provided evidence that these are involved in the tissue-specific processing of prohormones and neuropeptides. PC2 is expressed at high levels in the islets of Langerhans, where it participates in the processing of proinsulin to insulin (S.P.S. and D.F.S., unpublished data). To evaluate the regulated expression of the human PC2 (hPC2) gene we have analyzed its structure and characterized its promoter. A map of the gene was constructed by using 11 clones isolated from two human genomic DNA libraries. The gene spans >130 kilobase pairs and consists of 12 exons. Comparison with the structure of the gene encoding human furin, another member of this superfamily, revealed a high degree of conservation of exon–intron junctions. The hPC2 gene was localized to chromosome 20, band p11.2. The 5' flanking region of the hPC2 gene is very G+C-rich and contains six potential Sp1 binding sites but no TATA or CAAT box. Expression of chloramphenicol acetyltransferase reporter fusions containing the putative promoter region was observed to occur in β TC-3 mouse insulinoma cells but not in HepG2 human hepatoma cells, consistent with the known tissue-specific pattern of expression of the hPC2 gene. Analysis of the level of chloramphenicol acetyltransferase activity with several deletion mutants identified the region from –1100 to –539 from the translation start site as essential for hPC2 promoter activity.

Proteolytic processing at paired basic amino acids represents an important step in the maturation of a large number of prohormones, neuropeptides, and other biologically active peptides and proteins (1, 2). The search for the proteases responsible for this intracellular processing activity had led to the implication of various members of virtually all the known cellular protease families over the past two decades, but without any direct proof of their participation (1–4). The Kex2 protease of the yeast *Saccharomyces cerevisiae* was the first endoprotease shown unequivocally to be involved in processing under normal physiological conditions *in vivo* (5). Kex2 is a Ca^{2+} -dependent serine protease with a bacterial subtilisin-like catalytic domain that cleaves the pro- α -factor and pro-killer toxin proteins at dibasic sites (6, 7). In earlier experiments, we were unable to restore Kex2-like activity by expressing cDNA libraries from mammalian neuroendocrine tissues in Kex2-deficient strains of yeast (S.P.S., and D.F.S., unpublished data), but a subsequent PCR-based search for homologues of Kex2 resulted in the identification of a cDNA (PC2) that encodes a protein structurally related to Kex2 from a human insulinoma (8, 9). Analysis of the PC2 cDNA revealed that it encodes a 638-amino acid protein containing a 282-residue domain that is homologous to the catalytic

modules of both Kex2 and the bacterial subtilisins. Within this region 49% and 27% of the amino acids are identical to those in the aligned Kex2 and subtilisin BPN' sequences, respectively, and the catalytically important aspartic, histidine, and serine residues are all conserved. While our work on PC2 was in progress, a database search for proteins with significant homology to Kex2 revealed another putative member of this superfamily, the human *fur* gene product, furin (10). Furin was originally discovered in 1986 by Roebroek *et al.* (11) due to the proximity of its gene upstream to that of the *c-fes/fps* oncogene. Northern analyses have demonstrated that furin mRNA is present in a wide variety of tissues, including liver and kidney at high levels (12, 13). In contrast, PC2 mRNA is present in pancreatic islets, AtT-20 pituitary cells, and brain but is very low or absent in most other tissues, such as liver, spleen, and kidney (8, 9).

More recently, we and others have identified another cDNA encoding a protease related to PC2 (PC3/PC1) in islets and AtT-20 cells (14, 15). Like PC2, PC3 is expressed only in neuroendocrine tissues. However, the levels of PC2 and PC3 transcripts in various tissues appear to be inversely related. PC2 is expressed at high levels in pancreatic beta cells (8) and in the intermediate lobe of the pituitary (15), while PC3 is expressed at a relatively high level in the anterior pituitary (9) and at lower levels in pancreatic beta cells (8). PC2 and PC3 participate in the processing of proopiomelanocortin and proinsulin at paired basic amino acid sequences when these precursors are coexpressed with the proteases by vaccinia virus vectors in BSC-40 or COS-7 cells (refs. 16 and 17; S.P.S., G. Thomas, and D.F.S., unpublished data).

To investigate the regulation of the human PC2 (hPC2) gene, we needed to characterize its structural and promoter regions. Furthermore, the availability of the gene structure would allow comparisons to be made with other related genes and provide a basis for studies of potentially defective PC2 genes in man. Here, we report the structure and chromosomal localization of the hPC2 gene and data on the characterization of its promoter.¶

MATERIALS AND METHODS

Isolation of the hPC2 Gene. Human genomic DNA libraries obtained from Clontech, catalogue no. HL 1006d, and Stratagene, catalogue no. 946203, were screened with the hPC2 cDNA labeled by nick-translation (Amersham). Six and 5 independent clones were identified from the Clontech and Stratagene libraries, respectively. These 11 clones were

Abbreviations: RACE, rapid amplification of cDNA ends; CAT, chloramphenicol acetyltransferase; hPC2, human PC2.

§To whom reprint requests should be addressed at: Howard Hughes Medical Institute, The University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M90702).

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.

FIG. 1. Map of the hPC2 gene. The 12 exons are indicated. The translational initiation site is the 0 coordinate. The vertical lines in each clone represent natural *EcoRI* sites. Since sequence overlaps were not identified within introns 1, 2, 5, 9, and 11, the size shown is the minimal estimated size. kb, Kilobases.

characterized in detail by restriction mapping. Sequencing of genomic fragments subcloned into M13 or pGEM plasmids was carried out by the dideoxynucleotide method (18) using either universal or specific primers.

Rapid Amplification of cDNA Ends (RACE). This technique (19) was used to obtain PC2 cDNA with extended 3' and 5' ends. Primers used were as follows: 5' RACE, 5'-GCCTTC-CACTGGGAGACACA-3'; 3' RACE, 5'-AGCTTCACTCAC-TCACTGTCAATG-3' and 5'-AGTGGACCCTGATGCTG-CATG-3'. The 3'- and 5'-RACE reaction products were subcloned into the *HincII* site of pGEM-4Z (Promega), identified by colony hybridization, and sequenced.

Primer Extension. For primer extension analysis, a 20-residue oligonucleotide (5'-ATGTGGGAGCTGTGTGTAC-3') complementary to nucleotides -232 to -251 relative to the translation start site of the hPC2 gene was synthesized. This oligonucleotide was labeled by using T4 polynucleotide kinase and [γ - 32 P]ATP, annealed to 40 μ g of total human insulinoma RNA, and extended with avian myeloblastosis virus reverse transcriptase (GIBCO/BRL). The products were analyzed by electrophoresis in 5% polyacrylamide/8 M urea gels.

Fluorescence *in Situ* Chromosomal Hybridization. The analysis was carried out as described (20), with the hPC2 clones λhPC2-4 and -14 as probes.

Construction of Plasmids, Transfection, and Measurement of Chloramphenicol Acetyltransferase (CAT) Activity. The hPC2 5'-region constructs were prepared by ligation of fragments into the bacterial CAT reporter gene plasmid pCAT-Basic or

pCAT-Enhancer (containing a simian virus 40 enhancer element) (Promega). β TC-3 mouse insulinoma cells (21) or HepG2 human hepatoma cells (22) were transiently transfected using 5 μ g of the plasmid DNA and 250 μ g of Transfectam (Promega) per 60-mm dish (23). The plasmids were cotransfected with the β -galactosidase expression plasmid pSV- β -Galactosidase (Promega) as an internal control. After 48 hr the cells were harvested and CAT and β -galactosidase assays were performed (24, 25). Observed CAT activity was normalized with respect to β -galactosidase activity.

RESULTS

Structure of the hPC2 Gene. A map of the hPC2 gene was constructed from 11 genomic DNA fragments (Fig. 1). The gene consists of 12 exons and spans >130 kb. The sizes of the exons and introns are summarized in Table 1. The boundaries of the exon-intron junctions conform to the GT-AG rule (26). The catalytic domain is encoded by exons 3-11 (Fig. 2A). Several important structural features of the hPC2 protein are encoded within discrete exons. For example, the predicted signal peptide (exon 1), and the four catalytically important amino acid residues Asp¹⁶⁷, His²⁰⁸, Asp³¹⁰, and Ser³⁸⁴ are all found on separate exons. Although the sequences of the exons generally match the cDNA sequence previously reported (8), nucleotides 847-852, which were reported to be GACGTC, encoding Asp²⁸³ and Val²⁸⁴, were found to be GAGCTC in the gene. Resequencing of the hPC2 cDNA

Table 1. Exon-intron organization of the hPC2 gene

Exon no.	Exon size, bp	Sequence at exon-intron junction		Intron size, kb	Codon interrupted
		5' splice donor	3' splice acceptor		
1	490	CGA AAG gtaagc.....ccacag	CTT CCC	>17	Lys ⁵⁹
2	105	CCC AGG gtgagt.....ttccag	GTA AAG	>13	Arg ⁹⁴
3	114	TAT CTG gtgagt.....taccag	ATC AAT	2	Leu ¹³²
4	109	GAT G gtgagt.....tcacag	GG ATT	6	Gly ¹⁶⁹
5	38	AAC TAT gtaagt.....tttcag	AAT GCC	>16	Tyr ¹⁸¹
6	77	AAC AG gtaaac.....ccaaag	C CAC	29	Ser ²⁰⁷
7	89	GCA G gtaagc.....ccacag	GC ATC	7	Gly ²³⁷
8	176	AAC AAG gtaagg.....ccccag	GGC CGC	18	Lys ²⁹⁵
9	216	GGT GTG gtgagc.....ttccag	GCA ACC	>4	Val ³⁶⁷
10	101	GCT AA gtatgt.....atccag	C CTG	9	Asn ⁴⁰¹
11	228	CCC TGA gtaagt.....cctcag	GAA AAT	>8	Glu ⁴⁷⁷
12	>696				

Sites at which introns interrupt the mRNA and protein sequence are indicated. Exon sequences are in uppercase letters, and intron sequences are in lowercase letters. Sizes of introns were estimated by restriction mapping. bp, Base pairs.

FIG. 2. Structure of the hPC2 gene. (A) Schematic diagram showing arrangement of exons. Intron positions are indicated by arrowheads. The catalytic domain is enclosed within the bold box. Diamonds represent potential glycosylation sites. UT, untranslated region. (B) Amino acid sequence alignment of hPC2 and human furin. Positions of introns are denoted by arrowheads. Catalytic domain (boxed) and membrane-spanning region (underlined) are indicated. Active-site catalytic residues are indicated by asterisks. Gaps introduced into the alignment are indicated by hyphens. The number of amino acids is indicated at the end of each line. The exon-intron junctions of the furin gene are from refs. 11 and 13; the first intron in the 5' untranslated region is not shown.

revealed that the cDNA sequence corresponds exactly with the gene sequence, indicating that amino acids 283 and 284 are Glu and Leu, respectively. This sequence is also identical to that reported for mouse PC2 (9). There are also several nucleotide differences in the 5' and 3' untranslated regions between the cDNA and the gene. It is unclear whether these represent errors or sequence variants.

Northern analysis indicated that there are two hPC2 transcripts of 5 kb and 2.8 kb which may be due to differences in

the length of the 3' untranslated region of the mRNA (8). Despite several attempts, we were able to isolate only the 3' end of the shorter transcript by 3' RACE. There is a variant polyadenylation signal (AATATA), which is identical to that in the mouse PC2 cDNA (9). However, the actual polyadenylation site in hPC2 mRNA appears to be 4 or 5 bp upstream of that identified in mouse PC2 mRNA.

Comparison of exon-intron junctions in the hPC2 gene with those of the furin gene shows rather striking homology (Fig. 2B). Out of 11 exon-intron junctions in the hPC2 gene, 10 are located at the same position in the furin gene, which contains 15 introns (11, 13). Within the catalytic domain the hPC2 and furin genes differ in only 1 out of 8 exon-intron junctions (one intron is missing in the furin gene).

5' End of the hPC2 Gene. The 5' region of the hPC2 mRNA was first investigated by the 5' RACE procedure, which showed that at least 264 nucleotides upstream from the translation start site in the mRNA corresponded to the sequence of the genomic DNA. Primer extension analysis using a primer within this sequence identified the major and minor transcription start site at -312 and -284 from the translation start site, respectively (Fig. 3). The nucleotide sequence of the 1166-bp region upstream from the translation start site, which encompasses the putative promoter, was determined (Fig. 3). The sequence of this region showed no TATA or CAAT boxes. The region upstream from the transcription start site is extremely G+C-rich. The average G+C content is about 80% over the first 250 nucleotides; within this region is an 82-nucleotide core (-465 to -384) that contains only 4 A or T residues (95% G+C content). Five of the six potential binding sites for the transcription factor Sp1 (GGGCGG or CCGCCC) were found in this region. Two inverted AP-2 sites, one cyclic AMP response element, and one inverted Pan-1 binding motif are also present (27-29). There are four and three tandem repeats of the sequences GGCCG and TGCGC, respectively. Additionally, (GT)_n (GA)_m repetitive elements are present from -800 to -749. Based on a study of one family (25 individuals) with maturity-onset diabetes of the young, this region does not appear to be polymorphic (K. Xiang, personal communication).

Chromosomal Localization. *In situ* hybridization of biotin-labeled fragments of the hPC2 clones to normal human metaphase chromosomes resulted in specific labeling only of chromosome 20. However, signal was detected at two chromosome bands on the short arm of this chromosome, p11.2 and p12. Specific labeling of 20p11.2 was observed on two (6 cells), three (9 cells), or all four (8 cells) chromatids of the chromosome 20 homologues in 25 cells examined, whereas signal was detected at 20p12 on one (5 cells), two (5 cells), or three (4 cells) chromatids in these cells. Similar results were obtained in a second hybridization experiment using this probe. Thus, these results suggest that the hPC2 gene is localized to chromosome 20, band p11.2.

CAT Assay. A *Bgl* II-*Not* I fragment (-1100 to +32) was excised from genomic clone λ hPC2-3 and inserted into the CAT reporter gene plasmid pCAT-Basic. The resulting plasmid (pBglCAT) was transiently transfected into β TC-3 and HepG2 cells. Expression of endogenous PC2 mRNA in β TC-3 cells (positive) and HepG2 cells (negative) was confirmed by Northern analysis (data not shown). The pBglCAT construct was active in the β TC-3 cells but inactive in HepG2 cells (Fig. 4B). However, the *Bgl* II-*Not* I region was active in HepG2 cells when it was fused to the simian virus 40 enhancer element (Fig. 4B). These data are consistent with the known tissue specificity of hPC2 expression. A set of plasmids with progressively larger upstream deletions in the hPC2 gene promoter was then constructed (Fig. 4A) and transfected into β TC-3 cells. The resulting CAT activities, reflecting the activities of the corresponding promoter regions, are shown in Fig. 4.

-1166 AACGTCCTCCAGGTCTGAGAATGCACCTCCCGCGGTGCCACCCGGCCGCTCTCTCTGAAGGAGATCTTGGAGGTGC
 -1086 AGCCAGGACTCTAGAGAGCTCTCTCCAGTCACCATCGCGGGTGGGGCGAGCCGGGTCTCTGCTGGCTTCCCCAGCC
 -1006 GCTTCGCTCCGACCTGCCTGGAGCCTACAAAAAAGGGCTCGTCTTGCACTCTCGCCCCCATGGATCACCCTAG
 -926 AAGGTGCTTTCCCAACCTCTGGTGCCAAGGAGAGCAAACCTCCCGGGGATTGCCCCAAAGTGGTGGGAGGAGGTT
 -846 AATATTGCTTTGTTATTTCGGATAAGGGGAGGCAAGTATGCCTTGGGTGTGTGTGTGTGTGTTGAGAGAGAGAGAAAGA
 -766 GAGAGAGAGAGAGAGAGAGGTGTTTTTGCAGCGGGAACAAGCAGGAAGAGGCACAGAGCCGGCGCCCTGCTCCCTCG
 -686 GGGCTCTGAGAGCGGCACACACCGTAATTCCTTCCAGAGCCTACAGATTCCACAAACAATGCATCACTCCGCCGCC
 -606 GCTGGCCTGATTACAGCTGGGGGATTTCTTGGGCGCGCGCGGCCAGGCTGAGATGGGGGAGCTCTCCGGTGTGAA
 -526 AGGGCGGGGGCGCGGGCTCGCTGCGCTGCGCTGCGCTGCGCTGCGCGCCAGAGGCGAGGGCGCCGGCGCGCGCGG
 -446 GCGGGGGTGGCGGGGGCGGCGG
 -366 ACTACGTCACCCGAGCTGGTGTCTCCCCAGCCCTAGGGAATTGGAGCTGAGGAGGAGCTGAAATGCAGATTTAGC
 -286 ATCAAGCAGACCTACACTGCTCTTTCTCTCCGTACACACAGCTCCCCACATTTCGACCCCTGCCCGCGCGCGGCG
 -206 CGCCTGACTGCACGGCTTCCCTCCAGCCAGATGCTGGAGAACACACACTGATTGCTGCTTCCAGAACCTGTTTCACT
 -126 CTCTTTCTCTATACAAAGATTTTTTAACTATATATAAGATTCTTTATTGACCCCTCCCTCCGAGTCCCTGCTC
 -46 CGCCAGCTGCGCGCTCTAGCACCCTTTTCACTCCCAAGAAGG +1 ATG AAG GGT GGT TGT GTC TCC CAG
 Met Lys Gly Gly Cys Val Ser Gln

FIG. 3. Sequence of the 5' flanking region of the hPC2 gene. Nucleotide numbering is relative to the first nucleotide of the codon for the initiating methionine. The major transcriptional start site (★), the minor start site (#), and the 5' end of the cDNA obtained by 5' RACE (○) are indicated. The oligonucleotide used for primer extension is noted by a dashed line. Potential binding sites for transcription factor Sp1 are boxed. Two putative AP-2 sites, a cyclic AMP response element (CRE), and a Pan-1 binding motif are shown. Four tandem repeats of the sequence GGCCG, three tandem repeats of TGCGC, and a (GT)_n(GA)_m repetitive element are underlined.

DISCUSSION

The hPC2 gene consists of 12 exons separated by 11 introns and is relatively long (>130 kb). The exon-intron organization of the hPC2 and human furin genes is especially highly

conserved within the catalytic domain, implying that these two enzymes have arisen from a common ancestral gene.

Interestingly, although amino acid sequence similarities between the two proteases are evident well beyond the limits of the well-conserved catalytic domain, extending up to

FIG. 4. Mapping of the 5' flanking region of hPC2 gene. (A) Structure of CAT reporter gene constructs and their relative CAT activities in βTC-3 cells. Nucleotide numbering corresponds to that in Fig. 3. (B) Autoradiogram illustrating CAT activity associated with each construct. pSV2CAT, a positive control having the simian virus 40 gene early promoter/enhancer; pSyntCAT, a positive control having the rat insulin I promoter/enhancer; pCAT-B and pCAT-E, pCAT-Basic and pCAT-Enhancer without insert.

residue 594 in hPC2 (Fig. 2B), the hPC2 gene lacks three introns that occur in the furin gene in this region, suggesting that furin is ancestral to PC2. Moreover, the lack of any sequence similarity to furin beyond residue 594 in hPC2 is consistent with the possibility that a splice donor site was lost (presumably between exons 14 and 15 in an ancestral furin-like gene), allowing read-through to a new termination site (within the intron) to occur. This change very likely resulted in the loss of the transmembrane anchor and may have allowed PC2 to assume a new subcellular localization—i.e., to process precursors in the secretory granules rather than in the trans Golgi compartment. Further studies on the evolutionary origins of this subtilisin-related protease superfamily indicate that PC2 and furin were already encoded in separate genes in the earliest stages of vertebrate evolution (S.J.C. and A. Oliva, unpublished data).

By using fluorescence *in situ* hybridization, we have localized the hPC2 gene to human chromosome 20, band p11.2. The results are consistent with those reported by investigators who used mouse cDNA probes for radiolabeled *in situ* hybridizations (20p11.1–p11.2) (30). The observation of a second site of specific hybridization at 20p12 raises the possibility that sequences homologous to those contained in the hPC2 probes are located in this chromosomal band. Because the genes encoding furin (13) and PACE 4, a recently described homologue of furin (31), are both located on chromosome 15 and the PC3 gene is located on chromosome 5 (30), any additional PC2-related gene on chromosome 20 would have to represent either a pseudogene or a novel member of the superfamily.

The strong resemblance of the promoter region of the hPC2 gene to those of many “housekeeping” genes is rather surprising. However, a similar organization has also been found in the promoters directing the expression of the nerve growth factor receptor gene (32), the 5-lipoxygenase gene (33), and the carboxypeptidase E gene (34). The results of the CAT assay suggest the presence of regulatory elements essential for tissue-specific PC2 gene expression. Also, the region from –1100 to –539 was identified as indispensable for hPC2 promoter activity. Further study will clearly be necessary to more precisely localize and define important regulatory elements within the PC2 promoter region that generate its specific expression in neuroendocrine cells.

We thank Paul Gardner for synthesis of oligonucleotides, Terri Reid for assistance with cell culture, Anthony A. Fernald for technical assistance, and Susumu Seino for helpful suggestions. We thank Graeme Bell and Bryan Crenshaw for their critical reading and helpful comments. Human insulinoma tissue used in this study was kindly provided by Drs. Krishna M. Pinnamaneni, Arthur O'Connor, and Bruce D. Ragsdale, Desert Samaritan Hospital and Health Center, Mesa, AZ. Florence Rozenfeld assisted in preparing this manuscript. This work was supported by Public Health Service Grants DK13914, DK20595, and CA40046 and by the Howard Hughes Medical Institute. M.M.L. is a Scholar of the Leukemia Society of America.

1. Docherty, K. & Steiner, D. F. (1982) *Annu. Rev. Physiol.* **44**, 625–638.
2. Andrews, P. C., Brayton, K. & Dixon, J. E. (1987) *Experientia* **43**, 784–790.
3. Schwartz, T. W. (1990) in *Molecular Biology of the Islets of Langerhans*, ed. Okamoto, H. (Cambridge Univ. Press, Cambridge), pp. 153–205.
4. Lindberg, I. & Hutton, J. C. (1991) in *Peptide Biosynthesis and*

- Processing*, ed. Fricker, F. D. (CRC, Boca Raton), pp. 141–173.
5. Julius, D., Brake, A., Blair, L., Kunisawa, R. & Thorner, J. (1984) *Cell* **37**, 1075–1089.
6. Mizuno, K., Nakamura, T., Ohshima, T., Tanaka, S. & Matsuo, H. (1989) *Biochem. Biophys. Res. Commun.* **159**, 305–311.
7. Fuller, R. S., Brake, A. J. & Thorner, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1434–1438.
8. Smeekens, S. P. & Steiner, D. F. (1990) *J. Biol. Chem.* **265**, 2997–3000.
9. Seidah, N. G., Gaspar, L., Mion, P., Marcinkiewicz, M., Mbikay, M. & Chrétien, M. (1990) *DNA Cell Biol.* **9**, 415–424.
10. Fuller, R. S., Brake, A. J. & Thorner, J. (1989) *Science* **246**, 482–486.
11. Roebroek, A. J. M., Schalken, J. A., Leunissen, J. A. M., Onnekink, C., Bloemers, H. P. J. & Van de Ven, W. J. M. (1986) *EMBO J.* **5**, 2197–2202.
12. Schalken, J. A., Roebroek, A. J. M., Oomen, P. P. C. A., Wagenaar, S. S., Debruyne, F. M. J., Bloemers, H. P. J. & Van de Ven, W. J. M. (1987) *J. Clin. Invest.* **80**, 1545–1549.
13. Barr, P. J., Mason, O. B., Landsberg, K. E., Wong, P. A., Kiefer, M. C. & Brake, A. J. (1991) *DNA Cell Biol.* **10**, 319–328.
14. Smeekens, S. P., Avruch, A. S., LaMendola, J., Chan, S. J. & Steiner, D. F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 340–344.
15. Seidah, N. G., Marcinkiewicz, M., Benjannet, S., Gaspar, L., Beaubien, G., Mattei, M. G., Mbikay, M. & Chrétien, M. (1991) *Mol. Endocrinol.* **5**, 111–122.
16. Thomas, L., Leduc, R., Thorne, B. A., Smeekens, S. P., Steiner, D. F. & Thomas, G. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5297–5301.
17. Benjannet, S., Rondeau, N., Day, R., Chrétien, M. & Seidah, N. G. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3564–3568.
18. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
19. Nishi, M., Chan, S. J., Nagamatsu, S., Bell, G. I. & Steiner, D. F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5738–5742.
20. Rowley, J. D., Diaz, M. O., Espinosa, R., Patel, Y. D., van Melle, E., Ziemins, S., Taillon-Miller, P., Licter, P., Evans, G. A., Kersey, J. D., Ward, D. C., Domer, P. H. & Le Beau, M. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9358–9362.
21. Efrat, S., Linde, S., Kofod, H., Spector, D., Delannoy, M., Grant, S., Hanahan, D. & Baekkeskov, S. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9037–9041.
22. Knowles, B. B., Howe, C. C. & Aden, D. P. (1980) *Science* **209**, 479–499.
23. Behr, J.-P., Demeneix, B., Loeffler, J.-P. & Perez-Mutul, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6982–6986.
24. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
25. Herbomel, P., Bourachot, B. & Yaniv, M. (1984) *Cell* **39**, 653–662.
26. Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. & Chambon, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4853–4857.
27. Mitchell, P. J., Wang, C. & Tjian, R. (1987) *Cell* **50**, 847–861.
28. Sassone-Corsi, P. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7192–7196.
29. Nelson, C., Shen, L.-P., Meister, A., Fodor, E. & Rutter, W. J. (1990) *Genes Dev.* **4**, 1035–1043.
30. Seidah, N. G., Mattei, M. G., Gaspar, L., Benjannet, S., Mbikay, M. & Chrétien, M. (1991) *Genomics* **11**, 103–107.
31. Kiefer, M. C., Tucker, J. E., Joh, R., Landsberg, K. E., Saltman, D. & Barr, P. J. (1991) *DNA Cell Biol.* **10**, 757–769.
32. Sehgal, A., Patil, N. & Chao, M. (1988) *Mol. Cell. Biol.* **8**, 3160–3167.
33. Funk, C. D., Hoshiko, S., Matsumoto, T., Radmark, O. & Samuelsson, B. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2587–2591.
34. Jung, Y.-K., Kunczt, C. J., Pearson, R. K., Dixon, J. E. & Fricker, L. D. (1991) *Mol. Endocrinol.* **5**, 1257–1268.