Vol. 186, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1992 Pages 293-300

CHARACTERIZATION OF MOUSE INHIBIN α GENE AND ITS PROMOTER*

Jyan-Gwo J. Su and Aaron J. W. Hsueh

Div. of Reproductive Biology, Department of Gynecology/Obstetrics, Stanford University Medical Center, Stanford, CA 94305-5317

Received May 19, 1992

SUMMARY: Inhibin suppresses the pituitary secretion of FSH but not LH. The two forms of inhibin are composed of a common α subunit linked to either a βA or a βB subunit. The mouse inhibin α gene was isolated and shown to have two exons spanning a 1.7 Kb intron. The proximal 5' flanking region has neither TATA and CAAT boxes nor GC-rich area. Using the 5' flanking region of mouse inhibin α gene linked to luciferase gene, transfection of rat granulosa cells indicated that the first 165 bp of the promoter region is required for basal expression. The mouse inhibin α genomic clone should be useful for analysis of hormonal control of inhibin α transcription and the generation of mice with targeted deletion of this gene.

Inhibins are glycoproteins that preferentially inhibit the secretion and synthesis of FSH but not LH. Inhibin is produced primarily by ovarian granulosa and testicular Sertoli cells (1), and is stimulated by FSH, LH and agents enhancing cellular cAMP levels. There are two forms of inhibin, designated as inhibin A (α-βA) and inhibin B (α-βB), which are composed of a common α subunit linked by two disulfide bonds to either a βA or a βB subunit. Interestingly, dimerization of two β subunits leads to the formation of activins which can stimulate FSH secretion and have diverse functions during development (1, 2).

cDNAs for the inhibin subunits have been isolated from several species (3-6). They are evolutionarily related to the transforming growth factor-β (TGFβ) superfamily (1), whose members regulate cell growth and differentiation. The genomic DNA for inhibin α has been isolated from human (7) and rat (8-10), and the chromosomal location of mouse and human inhibins has been determined (11). Here, we isolate and characterize the mouse inhibin α gene, and study the function of its promoter.

*Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Numbers M95525 and M95526.

0006-291X/92 $4.00

MATERIALS AND METHODS

Isolation and analysis of mouse inhibin α genomic clones Total RNA was extracted from granulosa cells of gonadotropin-treated immature mice. First strand cDNA was made using the avian myeloblastosis virus (AMV) reverse transcriptase (Seikagaku America, Inc., Rockville, MD). For polymerase chain reaction (PCR), specific primers with appended Eco RI and Sal I sites (5'-[ACGT][ACGT]GAATTC AGCTCCCTCGATGCCTTG-3' and 5'-[ACGT][ACGT]GTCGACCAGTGTTGTGTAATGAGG-3') flanking the coding region of the mature inhibin α subunit were designed based on the rat inhibin α cDNA sequence (3). A 403 bp PCR DNA product was subcloned into pSKII+ plasmid and sequencing analysis indicated 93% homology to rat inhibin α cDNA. The mouse inhibin α cDNA fragment was ^{32}P-labelled by the random-priming method and used to screen a mouse liver genomic lambdaGEM-11 phage library (Promega Co., Madison, WI). After screening of $1.4X10^6$ colonies, 11 positive clones were isolated. The two exons and 2.5 Kb of the 5' flanking region were sequenced.

Analysis of the transcription start site For primer extension studies, a synthetic oligonucleotide (5'ATAGTTCACTTGCCCTGATG3') complementary to the region around the translation start site was ^{32}P end-labeled by using [gamma-^{32}P]ATP and T4 polynucleotide kinase (Promega Co.). 10^5cpm ^{32}P-labeled primer and 3.5μg total RNA of the mouse granulosa cells were co-precipitated with ethanol. The pellet was resuspended in hybridization solution and incubated overnight at 30^0C (12). Subsequently, primer extension reaction was performed with AMV reverse transcriptase in a reaction mixture containing dNTPs and RNAsin for 90 min at 42^0C (12). The products were analyzed on a 6% polyacrylamide gel with the size marker from a sequencing reaction using the same primer. For RNase protection analysis, a ^{32}P-labeled cRNA probe, complementary to the promoter region from +86 to -928 bp, was made by using [α-^{32}P]CTP and T3 RNA polymerase (13). After overnight hybridization (at 50^0C) of the cRNA probe with mouse granulosa cell RNA, the unprotected RNA was digested by RNase A and RNAse T1 at 30^0C (12). The protected ^{32}P cRNA was analyzed on a 6% polyacrylamide gel.

Construction of reporter genes and transfection of granulosa cells A Xho I linker was inserted into the cloning site of the p19LUC plasmid (14) containing the firefly luciferase gene to produce the p19LUCxho vector. Varying lengths (-99 to about -6000 bp) of 5' flanking region of the mouse inhibin α gene with the same 3' end at +71 bp were inserted into the 5' end of the luciferase gene in p19LUCxho. The smallest promoter region (-99 to +71 bp) was obtained using PCR whereas the larger 5' flanking regions were derived from the genomic clone based on restriction enzyme sites. The vector pRSV-ß-gal, containing the lacZ gene coding ß-galactosidase driven by the Rous sarcoma virus long terminal repeat, was used to monitor transfection efficiency. The luciferase gene expression vector pGL2-promoter (Promega Co.) driven by SV40 early promoter was used as a positive control.

Granulosa cells were obtained from immature estrogen-treated Sprague-Dawley female rats, as previously described (15). Cells were cultured in medium 199 (Gibco, Grand Island, NY) supplemented with 0.1%BSA, antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin sulfate) and 200mM L-Glutamine. About $5x10^5$ viable cells were plated per 10 X 35mm culture dish (Falcon, Cockeysville, MD) and incubated at 37^0C in a humidified 95% air/5% CO_2 atmosphere. Cells were plated for 2 h before transfection. Using the calcium phosphate precipitation method (16), cells were co-transfected with 0.7 pmole of plasmids expressing luciferase and 0.23 pmole of pRSV-

ß-gal for 4 h at 37^{0}C. Cells were rinsed twice with culture medium and incubated for different times with or without forskolin.

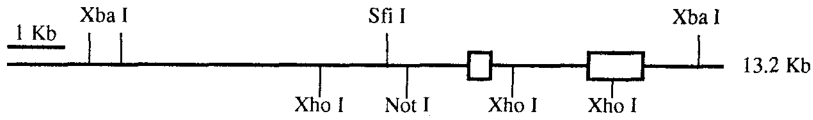
Assay of luciferase and ß-galactosidase activity After incubation, transfected cells were collected and lysed. Using a Monolight 2010 luminometer (Analytical Luminescence Lab., San Diego, CA), luciferase activity (relative light units: RLU) in the cell lysate was detected by mixing supernatant with the assay buffer (Promega Co.). After measurement of ß-galactosidase activity (17), the function of inhibin α promoter is expressed as the ratio of RLU/ß-galactosidase activity. Data represent the mean ± SEM of duplicate dishes of a representative experiment with each experiment repeated at least three times.

RESULTS AND DISCUSSION

Isolation and characterization of mouse inhibin α genomic clones The PCR-derived mouse inhibin α cDNA fragment was used as a probe to screen the mouse genomic library. Based on restriction enzyme mapping and Southern blot analysis, all of the positive clones correspond to three independent phages with inserts of 11.5, 12.7, 13.2 Kb. These clones have a common 5' end but with different 3' ends. The longest clone (Fig. 1A) includes 8.5 Kb 5' flanking region, two exons spanning an intron and a 1.7 Kb 3' flanking region. The nucleotide and amino acid sequence of the mouse inhibin gene with its flanking regions are shown in Fig. 1B. The intron-exon splice junctions are conserved among mouse, rat (9) and human genes (7). The intron size of mouse inhibin α is 1.7 Kb, similar to those found in human (7) and rat (8). The deduced protein sequence of mouse inhibin α is 95, 81, 83 and 78% homologous to its rat (3), bovine (4), porcine (5), and human (6) counterparts, respectively. However, there is one amino acid difference (residue 165) between our mouse genomic clone and a mouse testis inhibin α cDNA fragment (18). At 174 bp downstream from the stop codon, there is an AATAAA sequence (Fig. 1B) that may act as the polyadenylation signal.

Analysis of transcription start site and the promoter region Using primer extension analysis (Fig. 2), we identified five contiguous transcription start sites in mouse inhibin α promoter. The location of the middle and major site (assigned as "+1") is 71 bp 5' to the translation start site. Likewise, five protected fragments were found in RNase protection analysis with a major band 69 bp 5' to the translation start site (data not shown). The location deviation (2 bp) between the two methods may be related to the different migration patterns of RNA and DNA in the sequencing gel.

A



B

```
                 .      +1      .        .         .         .        50        .        .
 -19  CAGTGGGATGGGGAGGGGACAGTGGGGAGGTCCTAGACAGAAAGGGCACAGGGCAGCGTGTGGGTTCCCTGTCATCAGGGCAAGTGAACT

                 .      90      .        .         .         .       140        .        .
  72  ATGGTGAGCCAGCGGTCTCTGCTGCTCCTTTTGCTGTTGACCCTACGGGATGTGGACAGCTGCCAGGGGCCCGAACTTGTCCGGGAGCTC
   1  MetValSerGlnArgSerLeuLeuLeuLeuLeuLeuLeuThrLeuArgAspValAspSerCysGlnGlyProGluLeuValArgGluLeu

                 .     180      .        .         .         .       230        .        .
 162  GTCCTGGCCAAGGTGAAGGCTCTATTCCTAGATGCCTTGGGGCCCCCAGCAATGGATGGGGAAGGTGGGGATCCTGGAATAAGGCGTCTG
  31  ValLeuAlaLysValLysAlaLeuPheLeuAspAlaLeuGlyProProAlaMetAspGlyGluGlyGlyAspProGlyIleArgArgLeu

                 .     270      .        .         .         .       320        .        .
 252  CCTCGAAGACATGCCGTTGGGGGTTTCATGCACAGGACCTCTGAACCAGAGGAGGAAGATGTCTCCCAGGCTATCCTTTTCCCAGCTACA
  61  ProArgArgHisAlaValGlyGlyPheMetHisArgThrSerGluProGluGluGluAspValSerGlnAlaIleLeuPheProAlaThr

 342  Ggtaatgaagttggggattggcaggtcaactttgaagagtagccggtacctacttcatgg--------- 1.7kb intron -------
  91  G
                                                         .         .         .       380        .        .
      -ctcttttctttttctgcctgtcccctgcagGTGCCACCTGTGAGGACCAGCCAGCTGCCAGAGGGCTTGCCCAGGAGGCTGAGGAAGGC
                                                   lyAlaThrCysGluAspGlnProAlaAlaArgGlyLeuAlaGlnGluAlaGluGluGly

                 .     420      .        .         .         .       470        .        .
 402  CTCTTCACTTATGTATTCCGGCCATCCCAACACATACGCAGCCACCAGGTGACTTCAGCCCAGCTGTGGTTCCACACAGGCCTAGGCAGG
 111  LeuPheThrTyrValPheArgProSerGlnHisIleArgSerHisGlnValThrSerAlaGlnLeuTrpPheHisThrGlyLeuGlyArg

                 .     510      .        .         .         .       560        .        .
 492  AAGAGCACAGCAGCCGCCAATAGCTCTGCGCCCCTGCTAGATCTTCTGGTGCTATCATCTGGGGGGCCCATGCGTGTGCCTGTGTCCTTG
 141  LysSerThrAlaAlaAlaAsnSerSerAlaProLeuLeuAspLeuLeuValLeuSerSerGlyGlyProMetArgValProValSerLeu

                 .     600      .        .         .         .       650        .        .
 582  GGACAGGGCCCGCCACGCTGGGCTGTACTGCACCTGGCGGCGTCTGCTTTCCCTCTGCTGACCCACCCCATCCTGGTGTTGCTGCTGCGA
 171  GlyGlnGlyProProArgTrpAlaValLeuHisLeuAlaAlaSerAlaPheProLeuLeuThrHisProIleLeuValLeuLeuLeuArg

                 .     690      .        .         .         .       740        .        .
 672  TGCCCACTCTGTTCCTGCTCGGGCCGGCCTGAGACCACTCCTTTCCTGGTAGCCCACACTAGGGCTCGAGCACCCAGTGCGGGGGAGAGG
 201  CysProLeuCysSerCysSerGlyArgProGluThrThrProPheLeuValAlaHisThrArgAlaArgAlaProSerAlaGlyGluArg

                 .     780      .        .         .         .       830        .        .
 762  GCCCGGCGTTCAACTCCCTCCGTGCCTTGGCCTTGGTCTCCTGCGGCTTTGCGCTTGCTGCAGAGGCCTCCCGAGGAACCCGCTGCGCAT
 231  AlaArgArgSerThrProSerValProTrpProTrpSerProAlaAlaLeuArgLeuLeuGlnArgProProGluGluProAlaAlaHis

                 .     870      .        .         .         .       920        .        .
 852  GCCTTCTGCCATCGAGCTGCTCTCAATATCTCCTTCCAAGAGCTGGGCTGGGACCGCTGGATCGTACACCCTCCCAGTTTCATCTTCCAC
 261  AlaPheCysHisArgAlaAlaLeuAsnIleSerPheGlnGluLeuGlyTrpAspArgTrpIleValHisProProSerPheIlePheHis

                 .     960      .        .         .         .      1010        .        .
 942  TACTGCCATGGTAGCTGCGGGATGCCCACATCTGACCTGCCCCTGCCAGTCCCTGGGGTGCCCCCTACCCCGGTTCAGCCCCTGTTTTTG
 291  TyrCysHisGlySerCysGlyMetProThrSerAspLeuProLeuProValProGlyValProProThrProValGlnProLeuPheLeu

                 .    1050      .        .         .         .      1100        .        .
1032  GTGCCAGGGGCCAAGCCCTGCTGTGCTGCTCTTCCGGGGAGCATGAGGTCCCTACGCGTCCGAACCACCTCAGATGGAGGTTACTCTTTC
 321  ValProGlyAlaLysProCysCysAlaAlaLeuProGlySerMetArgSerLeuArgValArgThrThrSerAspGlyGlyTyrSerPhe

                 .    1140      .        .         .         .      1190        .        .
1122  AAGTATGAGATGGTACCCAACCTTATTACTCAACACTGTGCTTGTATCTAAAAGCACCTCCGCCTCCTCCTCCCATAGCCGATGGCCACA
 351  LysTyrGluMetValProAsnLeuIleThrGlnHisCysAlaCysIleEnd

                 .    1230      .        .         .         .      1280        .        .
1212  GTCAATTCACCACCCCAGGGTCCTCAGCTAGGAGGAGGACAGAGTGTGGAAAGTAGACAGTTTCCACTTCCTTTTCCCTACATCTTTCAG

                 .    1320      .        .         .         .      1370        .        .
1302  TATGAGGGATCCATATCCTGCTCCACCCAGGTCCTGTGGATAACAATAAAAAGGAAGTGTGTGTGCCTTTGTATGTGTTCCCCTCACGT

                 .    1410      .        .         .         .      1460        .        .
1392  CTTTGACAATGGGGTTGGGGAGGTCTGGGGTCAGAGAGAATTGCGTTGTGGGATTTTGAGTTAACTGCTTTTGGCTTTAGAGATCGACAG

              1490
1482  TCTAAGAGGTAAAA 1494
```

Figure 1. Schematic diagram of the mouse inhibin α gene and its nucleotide and deduced amino acid sequences. (A) Structure of the largest inhibin α genomic clone is shown with unique restriction enzyme sites. The open boxes indicate exons. (B) Nucleotide and protein sequences of the two exons with flanking regions are shown. The potential polyadenylation site is underlined. Nucleotides typed in small letters are intron sequences.

DNA sequencing analysis of the promoter region indicates that, as in the rat (8-10), the mouse inhibin α has neither TATA and CAAT boxes, nor GC-rich sequences (Fig. 3). In the 5' flanking region

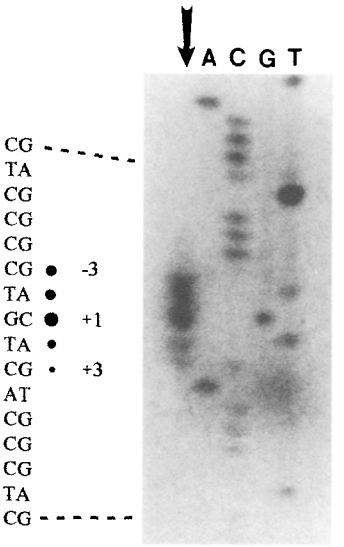


Figure 2. Transcription start sites of mouse inhibin α gene. In primer extension analysis, five contiguous transcription start sites, on the lane indicated by arrow, are shown together with the corresponding nucleotide sequences (dotted sequences on the left). The middle and major start site is assigned as +1.

```
    -700      -690      -680      -670      -660            -650      -640      -630      -620
Mouse CCTGTTCTTGGGAGTCTCTCTTTCCAGCCCCCTCCCACAGCCTGGGC-------CTCTTTCTCCTCCCTCCCTCCCA-CTCCGGCCTCCA
Rat   ....C....A....C.....---..... CTCTGGG.....C...-..........C....A..AG...

          -610      -600      -590      -580      -570      -560      -550      -540      -530
Mouse GGTCTCCAACTGCGGGCCTGGCTTGGGCTTGAGTGACTTTGTCGCCTGCAGCTGCCAATCCCAAAAATATTCTCTGATGACACAGCTGGA
Rat   ............C....A.........C.....................................................

          -520      -510      -500          -490      -480      -470      -460      -450
Mouse GGACAAGAGCTCAGACTGGCTCCTCCAGGCTAAGT----ATAGAGCAGGCGGGACCACCTGCCCTCGGCCCAGAGCCTCAACCCTAGGTC
Rat   ..T.......C.....................ATAG..........A.............CA.............C.....

      -440      -430      -420      -410      -400      -390      -380      -370      -360
Mouse TACCCTCCCACGTTGGCCTAGCAGCTGCTAGGATTGAAAAGAGCGGCAGAAAGTCTGAGGGGGGTGGTGCATTCTGTCCTCCTGACAAAT
Rat   .T...........................C.G..........CC.............TT...G.....................

      -350      -340      -330      -320      -310      -300      -290      -280      -270
Mouse GATTTGATGACTGGTCAACCTTAAGCACCCAGGCACCTGTGGTACCTGATGTAGATTCTATGTGTGTGTGTGTGTGTGTGTGTGTG
Rat   ............C.....C.........----------.....

      -260      -250           -240      -230      -220      -210      -200      -190
Mouse TGTGTGTGTGTG------------TATTTGAGGAGTGGGTTGTATGCTTGCCTGAATGGGTCAGGTCACTTGGGGTGAATACCCATCA
Rat   ............TGTGTGTGTGTG----------.....

        -180      -170      -160      -150      -140      -130      -120      -110      -100
Mouse CAGCCCCTTCCCCCACATTCTTGGCGGGAGTGGGAGATAAGGCTCAGGGCCACAGACATCTGCGTCAGAGATAGGAGGTCTCAATGCCAT
Rat   ..............................-.....

         -90       -80       -70       -60       -50       -40       -30       -20       -10
Mouse GGGCAGGGGCCACTGGGACTGTGGGGCGTGGGAAGGACTGGGGGAGACTGGGGTGAGAAGGGTAGAAGAAGGCCAGCAGTGGGATGGGGA
Rat   ..........A..........GA.............................-A.................A.............

           1        10        20        30        40        50        60        70 Met
Mouse GGGGACAGTGGGGAGGTCCTAGACAGAAAGGGCACAGGGCAGCGTGTGGGTTCCCTGTCATCAGGGCAAGTGAACTATG
Rat   ...A.G.....................................A.......C........G..........A.......
```

Figure 3. Nucleotide alignment of the 5' flanking region of mouse and rat inhibin α gene. The 700 bp promoter regions of mouse and rat (9) inhibin α genes were compared. Dots indicate conserved sequences whereas hyphens indicate deleted nucleotides.

Table 1. Potential response elements in the inhibin α gene

CRE	CS:	TGACGTCA	AP3	CS:	GGGTGTGGAAAG
	α:	(-125)TG-CGTCA		α:	(-72)GGGcGTGGgAAG
AP1	CS:	TGAGTCAG	ERE	CS:	CWKGTCANNNTGWCCT
	α:	(-125)TGcGTCAG		α:	(-1386)CAcGTCAGCAccACCT
AP2	CS:	CCCCAGGC	GRE	CS:	GGTACANNNTGTYCT
	α:	(-327)aCCCAGGC		α:	(-378)GGTgCATTCTGTCCT
		(-507)CtCCAGGC			
kappaE2	CS:	CCACCTGCC	μEBP-E	CS:	TNNAKYNNKNNMTNATGA
(/Rev)	α:	(-479)CCACCTGCC		IgHE:	TGAATTGAGCACTGtTGA
				α:	(-376)TGCATTCTGTCCTCcTGA
CuE4.1	CS:	ACCACCTG			
(/Rev)	α:	(-480)ACCACCTG			

K indicates G or T; Y indicates C or T; M indicates A or C; W indicates A or T; N indicates A, C, G or T; CS indicates consensus sequence; /Rev indicates inverted sequences. ERE and GRE indicate estrogen and glucocorticoid response elements, respectively. The number inside parentheses indicates the location of the response elements. IgHE: immunoglobin heavy chain enhancer.

of mouse inhibin α, there exist several potential cis-acting elements (Table 1). With one bp mismatch to the consensus sequence, cAMP response element (CRE) at -125 bp is conserved with that of rat inhibin α promoter which has been shown to confer cAMP responsiveness (10). Identical to those in immuglobulin genes, binding sites for μEBP-E (19), kappaE2 (20), and CuE4.1 (21) are also found in inhibin α promoter, raising the possibility that these transcription factors may regulate inhibin α promoter. The mouse inhibin α promoter is similar to its rat counterpart (Fig. 3). However, 5' and 3' sequences flanking the GT repeat region is deleted in rat and mouse genes, respectively.

Basal activity of inhibin α gene promoter and its stimulation by forskolin in rat granulosa cells To verify the promoter function of the mouse inhibin α gene, different lengths of the promoter region ranging from about -6000 to -98 bp with the same 3'end at +71 bp of the transcription start site have been ligated to the luciferase reporter gene. These constructs were transfected into granulosa cells, and cell extracts were collected 5 h later. As shown in Fig. 4, about 6000 and 2526 bp of the promoter regions have similar activity in driving the reporter gene. In contrast, shorter promoter constructs containing 308 and 165 bp have higher basal activity. Furthermore, truncation of the promoter to 98 bp resulted in a drastic decrease in activity. The construct, αLUC(-308-+71) containing a CRE, was shown to respond to forskolin, an

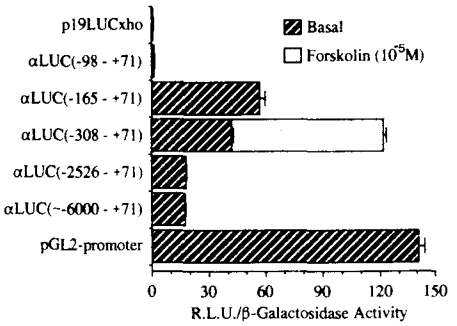


Figure 4. Basal activity and forskolin stimulation of the 5' flanking region of inhibin α gene. The indicated luciferase reporter gene constructs, containing different lengths of the inhibin α promoter, were co-transfected with pRSV-ß-gal into granulosa cells. The numbers inside the parentheses of αLUC indicate the 5' and 3' ends of the inhibin α promoter fragments in bp. p19LUCxho, a promoterless vector, is used as a negative control. Cells transfected with αLUC(-308-+71) were treated without or with forskolin (10^{-5} M). A reporter gene driven by the SV40 early promoter (pGL2-promoter) serves as a positive control. Data represent the mean ± SEM.

adenyl cyclase activator, with a 3-fold increase of promoter activity (Fig. 4). This result is consistent with that reported for rat inhibin α gene (10).

The isolated inhibin α gene promoter is functional in ovarian cells and responds to protein kinase A activation. The mouse gene should be useful for the study of cis-acting elements mediating the actions of FSH, activin and growth factors, hormones known to regulate inhibin α expression (15, 22). Mouse is extensively studied regarding its molecular genetics, and most advanced mammalian gene manipulation methods have been derived from this species. In addition to its use in directing reporter genes to inhibin α expressing tissues in transgenic animals, the present mouse inhibin α gene provides the possibility to perform targeted deletion of inhibin α gene using homologous recombination in embryonic stem cells (23). Generation of mutant mice with deletion of inhibin α gene should provide information on the physiological role of inhibin in reproduction and development.

Acknowledgments

We thank Dr. Chen-Ching Lai for technical advise and Dr. Håkan Billig for performing the computer analysis of nucleotide sequences. Plasmids p19LUC and pRSV-ß-gal were the generous gifts of Dr. David Brenner (UC San Diego).

REFERENCES

1. Vale, W.W., Rivier, C., Hsueh, A.J.W., Campen, C., Meunier H., et al. (1988) Recent Progress in Hormone Research 44,1-34.
2. Mathews, L.S., Vale, W.W., and Kintner, C.R. (1992) Science 255,1702-1705.
3. Woodruff, T.K., Meunier, H., Jones, P.B.C., Hsueh, A.J.W., and Mayo, K.E. (1987) Mol. Endocrinol. 1,561-568.
4. Forage, R.G., Ring, J.M., Brown, R.W., McInerney, B.V., Cobon, R.P., et al. (1986) Proc. Natl. Acad. Sci. USA 83,3091-3095.
5. Mason, A.J., Hayflick, J.S., Ling, N., Esch, F., Ueno, N., Ying, S.-Y., Guillemin, R., et al. (1985) Nature 318,659-663.
6. Mayo, K.E., Cerelli, G.M., Spiess, J., Rivier, J., Rosenfeld, M.G., et al. (1986) Proc. Natl. Acad. Sci. USA 83,5849-5853.
7. Stewart, A.G., Milborrow, H.M., Ring, J.M., Crowther, C.E., and Forage, R.G. (1986) FEBS Letters 206,329-334.
8. Feng, Z.M., Li, Y.P., and Chen, C.L. (1989) Mol. Endocrinol. 3,1914-1925.
9. Albiston, A.L., Lock, P., Burger, H.G., and Krozowski, Z.S. (1990) Mol. Cell. Endocrinol. 68,121-128.
10. Pei, L., Dodson, R., Schoderbek, W.E., Maurer, R.A., and Mayo, K.E. (1991) Mol. Endocrinol. 5,521-534.
11. Barton, D.E., Yang-Feng, T.L., Mason, A.J., Seeburg, P.H., and Francke, U. (1989) Genomics 5,91-99.
12. Ausubel, F.M., Brent, R., Kingston, R.E., et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York.
13. Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K., Green, M.R. (1984) Nucleic Acid Res 12,7035-7056.
14. van Zonneveld, A.-J., Curriden, S.A., and Loskutoff, D.J. (1988) Proc. Natl. Acad. Sci. USA 85,5525-5529.
15. Bicsak, T.A., Tucker, E.M., Cappel, S., Vaughan, J., Rivier, J. , Vale, W., et al. (1986) Endocrinology 119,2711-2719.
16. Chen, C., and Okayama, H. (1987) Mol. Cell Biol. 7,2745-2752.
17. Herbomel, P., Bourachot, B., and Yaniv, M. (1984) Cell 39,653-662.
18. Tone S, Katoh Y, Fujimoto H, Togashi, S., Yanazawa, M., Kato, Y., and Higashinakagawa T. (1990) Differentiation 44,62-68.
19. Peterson, C.L., Eaton, S., Calame, K. (1988) Mol Cell Biol 8,4972-4980.
20. Lenardo, M., Pierce, J.W., and Baltimore, D. (1987) Science 236,1573-1577.
21. Ephrussi, A., Church, G.M., Tonegawa, S., Gilbert, W. (1985) Science 227,134-140.
22. LaPolt, P.S., Soto, D., Su, J.-G., Campen, C.A., Vaughan, J., Vale, W., et al. (1989) Mol. Endocrinol. 3,1666-1673.
23. Frohman, M.A., and Martin, G.R. (1989) Cell 56,145-147.