Structure and Function of Human Prepro-orexin Gene*

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Orexins (orexin-A and -B) are recently identified potent orexigenic peptides that are derived from the same precursor peptide and are highly specifically localized in neurons located in the lateral hypothalamic area, a region classically implicated in feeding behavior. We cloned the whole length of the human prepro-orexin gene and corresponding cDNA. The human prepro-orexin mRNA was predicted to encode a 131-residue precursor peptide (prepro-orexin). The human prepro-orexin gene consists of two exons and one intron distributed over 1432 base pairs. The 143-base pair first exon includes the 5'-untranslated region and a small part of the coding region that encodes the first seven residues of the secretory signal sequence. The second exon contains the remaining portion of the open reading frame and 3'-untranslated region. The 3.2 kilobase pairs of the 5'-upstream region from a cloned human prepro-orexin gene promoter is sufficient to direct the expression of the Escherichia coli β-galactosidase (lacZ) gene in transgenic mice in the lateral hypothalamic area and adjacent regions. The lacZ-positive neurons were positively stained with anti-orexin antibody but not with anti-melanin-concentrating hormone antibody. These findings suggest that this genomic fragment contains all the necessary elements for appropriate expression of the gene and will be useful for the targeted expression of the exogenous gene in orexin-containing neurons. These mice might also be useful for examining the molecular mechanisms by which orexin gene expression is regulated.

Orexins (orexin-A and -B) are neuropeptides that were identified as endogenous ligands for an orphan G-protein-coupled receptor, which was originally found as an expressed sequence tag from human brain (1). Orexin-A and -B are derived from the same precursor peptide (prepro-orexin) by proteolytic processing. They bind and activate two closely related G-protein-coupled receptors, termed OX1 and OX2 receptors. OX1 receptor is selective for orexin-A, whereas OX2 receptor is a nonselective receptor for both orexin-A and -B. Prepro-orexin mRNA and immunoreactive orexin-A are highly specifically localized in neurons within and around the lateral hypothalamic area (LHA) in the adult rat brain, a region implicated in feeding behavior (2–4). Orexin-containing neurons diffusely innervate the entire brain, including monosynaptic projections, to various regions of the cerebral cortex, limbic system, and brain stem (5, 6). Orexins stimulate food consumption when administered intracerebroventricularly (1). Orexin gene expression is up-regulated upon fasting, suggesting the existence of molecular mechanisms that control orexin gene expression according to the nutritional status of the animal (1).

Expression of the orexin gene is highly restricted to neurons located in the LHA, indicating the existence of molecular mechanisms by which orexin gene transcription is highly specifically performed by the distinct population of neurons in these areas (1, 5, 7). Radiation hybrid mapping showed that the human prepro-orexin gene is located at human chromosome 17q21 (1). We have already reported that human prepro-orexin mRNA can also be exclusively detected in the hypothalamus/subthalamic regions (1). The mechanisms by which orexin gene expression is highly restricted to the distinct populations of neurons in these regions is of interest.

As the first step toward unveiling these mechanisms, we cloned fragments of the human prepro-orexin gene and its corresponding cDNA to determine their complete primary structures. One way to study the physiological roles of the orexin neuronal system would be to examine the consequences of expression of exogenous genes in orexin-producing neurons of transgenic mice, thereby manipulating the cellular environment in vivo. However, such studies require the use of an appropriate gene promoter to direct gene expression to orexin-producing neurons. Human prepro-orexin gene promoter is indeed a good candidate for targeting gene expression to orexin-producing neurons.

From these points of view, we made a prepro-orexin-lacZ fusion gene and tested it in transgenic mice to identify a DNA fragment containing all the necessary elements for appropriate orexin expression. This approach would also be useful to examine the mechanism by which orexin gene expression is highly restricted to the LHA and adjacent regions.

EXPERIMENTAL PROCEDURES

Cloning of Human Prepro-orexin Gene—Because we found that the full-length rat orexin cDNA, which contains CTG triplet repeats (encoding the oligo-leucine stretch in the signal sequence), tends to cross-hybridize with a number of unrelated genes, we used a 0.29-kb segment of rat cDNA encoding Glu23–Ser51 of prepro-orexin as a probe (1). Approximately one million clones from a human genomic library (CLONTECH) were screened by plaque hybridization with this cDNA probe. We isolated several clones, and one of the longest clones, 1

1 The abbreviations used are: LHA, lateral hypothalamic area; MCH, melanin-concentrating hormone; kb, kilobase pair(s); bp, base pair(s); PIPES, 1,4-piperazinediethanesulfonic acid; lacZ, E. coli β-galactosidase gene with a nuclear localization signal.
AghLig72–7, was analyzed further. We subcloned the 4.9-kb BamHI fragment of AghLig72–7 insert into the pBluescript SKII (+) vector (Stratagene), which we termed pghLig72-Bam, and subjected it to further characterization by standard procedures. DNA sequencing was performed with Thermo Sequenase (Amersham Pharmacia Biotech) and analyzed with LI-COR 4000L or IR4200 DNA sequencer following the manufacturer’s instructions.

Genomic Southern Blot Analysis—A membrane that contains human genomic DNA digested with designated restriction enzymes was purchased from CLONTECH (Human GENO-BLOT; catalog number 7700-1). The 544-bp PstI fragment of human prepro-orexin gene was labeled by the random priming method with [α-32P]dCTP to a specific activity of 5 × 108 cpm/mg and used as a probe. The membrane was incubated at 65 °C in a solution containing 1 M NaCl, 1% SDS, 150 mg/ml salmon sperm DNA, and 5 μg/ml probe. The membrane was then washed in a solution containing 0.1× SSC (1× SSC = 0.15 μM NaCl and 0.015 μM sodium citrate) and 0.1% SDS at 50 °C and subjected to autoradiography at −80 °C for 48 h.

Primer Extension Analysis—An infrared dye (IRD41)-labeled primer 5′-GTAGCCGGGAAAGGAGATGTCTGTGGTGG-3′, which is complementary to position 72 to 100 of human prepro-orexin gene, was hybridized to 1 μg of human whole brain poly(A)+ RNA (purchased from CLONTECH) in a solution containing 80% formamide, 400 mM NaCl, 10 mM EDTA, 40 mM PIPES (pH 6.4) at 30 °C. The hybridized RNA/primer was precipitated with ethanol and then subjected to reverse transcription with avian myeloblastosis virus reverse transcriptase. The same primer was also used for dideoxy sequencing reaction with Thermo Sequenase (Amersham Pharmacia Biotech) using AghLig72–7 DNA as a template. The samples were analyzed by 4000L DNA sequencer (LI-COR).

Cloning of Human Prepro-orexin cDNA—Human cDNA encoding prepro-orexin was cloned by the 3′-rapid amplification of cDNA ends reaction with cloned human prepro-orexin gene as a template with two oligonucleotide primers, 5′-GACCCGGCCATTCTTGGTG-3′ and 5′-AAGTGCACGGTGTCAGGGTCTCAGGGTGTT-3′. The product corresponded to position −118 to 122 of human prepro-orexin gene shown in Fig. 2 and had an artificial SalI site at the 3′-end. We digested this fragment with PstI and SalI and ligated it to the 1.6-kb PstI fragment of the cloned prepro-orexin gene (from −1.7 kb to 72 bp shown in Fig. 2). This DNA fragment was then ligated to BamHI-PstI (−1.35 to −1.7 kb) fragment of the gene. The resulting DNA fragment, which has a 3.15-kb 5′-flanking region and the whole length of the 5′-noncoding region, was used as the promoter that directs expression of the cloned Escherichia coli lacZ gene with an inserted SV40 antigen nuclear localization signal (nlacZ), derived from pnlacF (8). The 3′-end of nlacZ was ligated to murine protamine-1 (mPrm1) gene fragment (from +95 relative to the transcription start site to +625), which includes part of exon 1 and all of intron 1 and exon 2, including the poly(A)+ adenylation signal and site.

The resulting gene fragment free of vector sequence was isolated and injected into fertilized mouse eggs to generate transgenic founder animals. The presence and copy numbers of the transgene was identified by tail blot (9). Animals from these lines were examined by β-galactosidase histochemical technique to assess transgene expression in the tissues.

LacZ Histochemical and Immunohistochemical Staining—The mice were perfused via the heart with phosphate-buffered saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). Tissue fragments were further fixed for 60 min at 4 °C in the same fixative buffer. They were then rinsed three times with a solution containing 0.1 M phosphate buffer (pH 7.3), 2 mM MgCl2, 0.01% sodium deoxycholate, and 0.02% Nonidet P-40. The staining reaction was then performed by incubating the tissue fragments for 16–24 h at 37 °C in a solution containing 0.1 M phosphate buffer (pH 7.5), 2 mM MgCl2, 0.01% sodium deoxycholate, 0.02% Nonidet P-40, 1 mg/ml X-gal, 5 mM K4Fe(CN)6, and 5 mM K3Fe(CN)6. Post fixation was performed for 24–48 h in 10% formalin. Tissues were dehydrated and embedded in paraffin for sectioning.

For immunohistochemical staining, coronal sections of brain (40μm)
were incubated for 35 min in 0.6% hydrogen peroxide to eliminate endogenous peroxide activity. Sections were rinsed in phosphate buffer and incubated for 30 min in Tris-buffered saline containing 3% normal goat serum and 0.25% Triton X-100. Thereafter, sections were incubated with rabbit polyclonal anti-orexin antibody (1) or anti-melanin-concentrating hormone (MCH) antibody diluted 1/2000 in Tris-buffered saline containing 1% normal goat serum and 0.25% Triton X-100 overnight at 4 °C. The primary antibody was localized with the avidin-biotin system (Vector Laboratories). Bound peroxidase was visualized by incubating sections with 0.01M imidazole acetate buffer containing 0.05% 3,3′-diaminobenzidine tetrahydrochloride and 0.005% hydrogen peroxide.

RESULTS

Structure and Sequence of Human Prepro-orexin Gene and Its Transcript—By screening a human genomic library (constructed in EMBL3 SP6/T7 vector) with rat prepro-orexin cDNA probe, we cloned a recombinant λphage clone λghLig72–7, which has an approximately 18-kb insert that contains the whole length of human prepro-orexin gene. We subcloned the 4.9-kb BamHI fragment of λghLig72–7 insert into the pBluescript SKII(+) vector (Stratagene), which we termed pghLig72Bam, and subjected it to further analyses.

FIG. 2. Complete nucleotide sequence of human prepro-orexin gene cloned in pLig72Bam. The exon sequence is shown in capital letters, whereas introns and flanking sequences are shown in lowercase letters. Nucleotide residues are numbered positively, starting at the transcription initiation site determined by the primer extension analysis shown in Fig. 4 and negatively in the 5′-flanking sequence. Alu repeat elements are indicated by dashed underlines. The deduced amino acid sequence for prepro-orexin is shown under the nucleotide sequence of the translated region. Amino acid residues are numbered beginning with the initiation methionine residue. The sequences of mature orexin-A and orexin-B are underlined. The consensus sequence for the branch site of the intron is double-underlined. The poly(A) signal (AATAAA) is indicated by thin underline. The nucleotide sequence has been submitted to GenBank® (accession number AF118885).
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Fig. 3. Southern blot analysis of human DNA with cloned human prepro-orexin gene fragment as a probe. Each lane contains 5 μg of digested human genomic DNA. The membrane was hybridized with a 544-bp PstI fragment of ρghLig72–7 insert. The positions of HindIII fragments of λ-DNA are shown as molecular weight markers.

Fig. 4. Mapping of sites of transcription initiation by primer extension method. An infrared dye (IRD41)-labeled primer (5′-GTAGCCGGGAAAGGAGATGTCTGTGGTGG-3′) was hybridized with 1 μg of human brain poly(A) RNAs or 30 μg of yeast tRNA. The hybridized primer/RNA was extended with reverse transcriptase. The same primer was used for DNA sequencing with Thermo Sequenase using ρghLig72–7 as a template. These samples were analyzed simultaneously by a DNA sequencer (LI-COR). Asterisks show the transcription start site.

Potential site for the promoter region was predicted to start at position -291 by BCM Gene Finder web site. This program also ignores the TATAAA sequence in position 5–10.

The restriction enzyme map and the structural organization of the human prepro-orexin gene are schematically shown in Fig. 1. The complete nucleotide sequence of the structural gene and the 5′- and 3′-flanking regions determined from the results of primer extension analysis and the cDNA sequence are shown in Fig. 2. The ρghLig72-Bam insert contains a 3149-bp 5′-flanking region, a 1432-bp structural gene, and a 364-bp 3′-flanking region. The structural gene consists of two exons (143 and 473 bp) and one intron (816 bp). Southern blots of human genomic DNA were probed with the 544-bp PstI fragment of ρghLig72–7, which contains a part of intron 1 and exon 2. Single EcoRI (>23 kb), HindIII (7.0 kb), BamHI (4.9 kb), and PstI (0.55 kb) fragments of the genomic DNA hybridized with the probe, indicating that the cloned DNA is an authentic copy of the genomic DNA (Fig. 3).

The intron 1 starts and ends with the consensus sequence for the 5′-end (KAG/GTRA) and 3′-end (YnNYAG/G, n = 10–12) of U2-type GT-AG introns (10), respectively, and has a putative branch site (CTRAY) at nucleotides 549–553 (CTGAT) (Fig. 2).

A BLAST (blastn) search of the GenBank data bases with the sequence presented in Fig. 2 failed to find a significantly similar sequence, except for several highly repetitive elements of the primate Alu family (nucleotides -2414 to -2143, -1916 to -1640, -1627 to -1343, -1292 to -1004, and -885 to -613) (searched with CENSOR Web Server). These regions show 64–85% nucleotide identity with the consensus sequence of human Alu repeat.

Comparison of the gene and cDNA sequences, together with the results form the primer extension analysis (Fig. 4), suggest that human prepro-orexin mRNA, which is 616 nucleotides long excluding the poly(A) tail, is encoded by two exons distributed over 1432 base pairs of the human genome (Figs. 1 and 2). The 5′-most ATG codon of the cDNA (nucleotides 123–125) was preceded by an in-frame stop codon (TGA; nucleotides 108–110), and the sequence around this initiation codon conformed well to Kozak’s rules (11). The open reading frame starting with this ATG encodes a 131-residue polypeptide, human prepro-orexin (Fig. 2). The 5′-untranslated region and the first 7 residues of the secretory signal sequence correspond to the 143-bp first exon. The 473-bp second exon contains the remaining portion of the open reading frame and the 102-bp 3′-untranslated region. Thus, the remaining portion of the signal sequence (residues 8–33) and pro-orexin are encoded in exon 2 (Fig. 2).

Structure and Sequence of Human Prepro-orexin—The first 33 amino acids of human prepro-orexin exhibited characteristics of a secretory signal sequence: a hydrophobic core followed by residues with small polar side chains (12). The SignalP Server web site predicted that Ala33–Gln34 was the most likely site for signal sequence cleavage. The orexin-A sequence starts with Gln34, which is presumably cyclized enzymatically into the N-terminal pyroglutamyl residue by transamidation (13, 14). Thus, the mature peptide directly follows the signal pep-

http://defrag.bcm.tmc.edu:9503/genefinder/gf.html.

http://charon.lpi.org/~server/censor.html.

http://www.cbs.dtu.dk/services/SignalP/.
Orexin were stained by generally, only 30–50% of neurons containing immunoreactive gene were only a subset of the neurons expected to stain. (Fig. 5). We observed that the neurons expressing the trans- necessary elements for appropriate expression in these regions (Table I), suggesting that this fragment contains all the specific expressed in the LHA/subthalamic region in two lines (Table I), confirming that this promoter does not direct expression to the MCH-negative controls (not shown). The section was then stained with anti-orexin antibody (golden brown). B, higher magnification of section shown in A. Note that all lacZ-positive neurons (stained blue) contain immunoreactive orexins (stained brown). C, the lacZ-stained section was doubly stained with anti-MCH antibody. D, higher magnification of section shown in C. Note that MCH neurons do not overlap with lacZ-positive neurons.

Expression of β-Galactosidase Gene in Transgenic Mice—A fragment of human prepro-orexin gene, which contains a 3.15-kb 5′-flanking region and the whole length of the 5′-noncoding region of exon 1, was fused to the modified E. coli lacZ gene, which has an SV40 T antigen nuclear localization signal (nlacZ) (8) (Fig. 1). We generated transgenic mice using this construct as a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene. A total of six lines bearing the transgene were examined, and this construct was a transgene.

We also examined whether MCH-containing neurons, which are also known to be exclusively located in the LHA and adjacent regions, express the transgene or not. As shown in Fig. 5, MCH-positive neurons did not show lacZ activity, suggesting that this promoter does not direct expression to the MCH neurons. We could not observe any positive lacZ staining in tissues outside the brain.

Expression of E. coli β-galactosidase gene in the brain of the human orexin-nlacZ fusion gene transgenic mice line A3 (Table I), visualized by lacZ histochemistry. The expression of transgene is observed in orexin-containing neurons (A and B) but not in MCH-containing neurons (C and D) in the LHA. A, visualization of neurons containing E. coli β-galactosidase directed by cloned human orexin gene promoter in the brain of transgenic mice. The coronal section of brain stained with lacZ histochemistry showing the bilateral and symmetrical distribution of labeled neurons within and around the LHA (stained blue). No detectable signal other than background was generated in transgene negative controls (not shown). The section was then stained with anti-orexin antibody (golden brown). B, higher magnification of section shown in A. Note that all lacZ-positive neurons (stained blue) contain immunoreactive orexins (stained brown). C, the lacZ-stained section was doubly stained with anti-MCH antibody. D, higher magnification of section shown in C. Note that MCH neurons do not overlap with lacZ-positive neurons.

These observations suggest that human orexin-A and -B are also C-terminally amidated like their counterparts in the rodent. The predicted human orexin-A sequence was identical to rodent/bovine orexin-A. Human orexin-B had two amino acid substitutions compared with the rodent sequence. Overall, the human prepro-orexin sequences were 83% identical to the rat counterpart (1). The majority of amino acid substitutions were found in the C-terminal part of the precursor, which appears unlikely to encode for another bioactive peptide.

Expression of β-Galactosidase Gene in Transgenic Mice—A fragment of human prepro-orexin gene, which contains a 3.15-kb 5′-flanking region and the whole length of the 5′-noncoding region of exon 1, was fused to the modified E. coli lacZ gene, which has an SV40 T antigen nuclear localization signal (nlacZ) (8) (Fig. 1). The neurons containing orexins (orexin neurons) identified as a recognition site for prohormone convertases (17). The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg. The last residue of the sequence of orexin-B, Met96 residue, is again followed by Gly-Arg-Arg.

Expression of β-Galactosidase Gene in Transgenic Mice—A fragment of human prepro-orexin gene, which contains a 3.15-kb 5′-flanking region and the whole length of the 5′-noncoding region of exon 1, was fused to the modified E. coli lacZ gene, which has an SV40 T antigen nuclear localization signal (nlacZ) (8) (Fig. 1). We generated transgenic mice using this construct as a transgene. A total of six lines bearing the transgene were examined, and lacZ expression could be detected in three of these (Table I).

The lacZ staining showed that the transgene was highly specifically expressed in the LHA/subthalamic region in two lines (Table I), suggesting that this fragment contains all the necessary elements for appropriate expression in these regions (Fig. 5). We observed that the neurons expressing the transgene were only a subset of the neurons expected to stain. Generally, only 30–50% of neurons containing immunoreactive orexin were stained by β-galactosidase histochemical technique. All of the lacZ-positive neurons in the LHA contained immunoreactive orexins (Fig. 5).

Line D5 showed ectopic expression of lacZ in several regions that do not express orexins, including the arcuate nucleus, periventricular nucleus, and preoptic nuclei (Table I). This ectopic expression was only observed in line D5 and might be because of a positional effect. We could not observe any ectopic expression of transgene other than the eutropic expression in the LHA in line A3 and line J2 (Table I).

We also examined whether MCH-containing neurons, which are also known to be exclusively located in the LHA and adjacent regions, express the transgene or not. As shown in Fig. 5, MCH-positive neurons did not show lacZ activity, suggesting that this promoter does not direct expression to the MCH neurons. We could not observe any positive lacZ staining in tissues outside the brain.

Discussion

Recent studies have identified several neuropeptide and receptor systems in the hypothalamus that are critical in the regulation of body weight (18). The LHA has long been considered essential in regulating food intake and body weight, because cell-specific lesions of this region can result in decreased food intake and body weight (2), and this region contains glucose-sensing neurons (4). We recently identified a family of neuropeptides, orexins, which are localized exclusively in neurons in the LHA. Orexins increase food intake when administered intracerebroventricularly. The neurons containing orexins (orexin neurons) diffusely innervate the entire brain, including monosynaptic projections to the cerebral cortex, limbic system, and brain stem (5–7). Therefore, orexin neurons may be ideally positioned to regulate cognitive, motivational, emotional, and autonomic aspects of food intake and body weight regulation.

Orexin neurons are shown to be highly specifically localized within and around the LHA in rodents and humans (1, 5–7). These observations suggest the existence of molecular mecha-
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Transgenic line D5 showed ectopic expression in several regions, which may be because of a positional effect (Table I). Alternatively, there may be other necessary elements to ensure the proper gene expression in other genomic regions. In this case, ectopic expression would be eliminated by the inclusion of additional DNA for the endogenous gene, leading to valuable insights into the regulatory mechanism of the endogenous gene.

In any case, the 3.2-kb human prepro-orexin promoter we used in this study was sufficient to direct expression of the exogenous gene in orexin-producing neurons in transgenic mice. Therefore, this promoter might be useful to examine the consequences of the expression of exogenous molecules in orexin neurons of transgenic mice, thereby manipulating the cellular environment in vivo. This genomic fragment will also be useful for the targeted ablation of orexin neurons by using it as a promoter that drives toxin expression (20).

Prepro-orexin mRNA was shown to be up-regulated under fasting conditions, indicating that these neurons somehow sense the nutritional status of the animal. We have found that orexin gene expression is influenced by plasma glucose and leptin levels.5 Human prepro-orexin lacZ transgenic mice will also be useful to examine the molecular mechanisms by which orexin gene expression is regulated.

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REFERENCES
1. Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R., M., Tanaka, H., Williams, S., C., Richardson, J. A., Kozlowski, G. P., Wilson, S., Arch J. R., Buckingham, R. E., Haynes, A. C., Carr S. A., Annan, R. S., McNulty, D. E., Liu, W. S., Terrett, J. A., Elshourbagy, N. A., Bergsma, D. J., and Yanagisawa, M. (1998) Cell 92, 573–585
2. Bernardis, L. L., and Bellinger, L. L. (1996) Neurosci. Biobehav. Rev. 20, 189–287
3. Bernardis, L. L., and Bellinger, L. L. (1993) Neurosci. Biobehav. Rev. 17, 141–193
4. Oumura, Y. (1980) In Handbook of the Hypothalamus, pp. 557–620, Marcel Dekker, Inc., New York
5. Date, Y., Ueta, Y., Yumashita, H., Yamaguchi, H., Matsukura, S., Kangawa, K., Sakurai, T., Yanagisawa, M., and Nakazato, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 748–753
6. Peyron C., Tighe, D. K., van den Pol, A. N., de Lecea, L., Heller, H. C., Sutcliffe, J. G., and Kudliff, T. S. (1998) J. Neurosci. 18, 9996–10015
7. Elias C. F., Saper C. B., Maratos-Flier, E., Tritos, N. A., Lee, C. Y., Kelly, J., Tatro, J. B., Hoffmann, G. E., Ollmann, M. M., Barsh, G. S., Sakurai, T., Yanagisawa, M. and Elmquist, J. K. (1998) J. Comp. Neurol. 402, 442–459
8. Mercer, E. H., Hoyle G. W., Kapur, R. P., Brinster R. L. and Palmiter, R. D. (1991) Nature 357, 703–716
9. Brinster, R. L., Chen, H. Y., Tyrnauer, M., Senear, A. W., Warren, R., and Palmier, R. D. (1981) Cell 27, 223–231
10. Sharp, P. A., and Burge, C. B. (1997) Cell 91, 875–879
11. Kozak, M. (1984) Nucleic Acids Res. 12, 857–874
12. von Heijne, G. (1986) Nucleic Acids Res. 14, 4683–4690
13. Bateman, A., Solomon, S., and Bennett, H. P. J. (1990) J. Biol. Chem. 265, 22130–22136
14. Busby, W. H., Jr., Quackenbush, G. E., Humm, J., Youngblood, W. W., and Kizer, J. S. (1987) J. Biol. Chem. 262, 8532–8536
15. Bradbury, A. F., and Smyth, D. G. (1991) Trends Biochem. Sci. 16, 112–115
16. Eipper, B. A., Milgram, S. L., Husten, E. J., Yun, H. Y., and Mains, R. E. (1993) Protein Sci. 2, 489–497
17. Rouille, Y., Dulac, S. J., Lund, K., Furuta, M., Gong, Q., Lipkind, G., Oliva, A. A., Jr., Chan, S. J., and Steiner, D. F. (1995) Front. Neuroendocrinol. 16, 322–361
18. Flier, J. S., and Maratos-Flier, E. (1998) Cell 92, 437–440
19. MacGowen, R., Campbell, R., Peterson, A., and Sapinza, C. (1989) Genes Dev. 3, 1669–1676
20. Palmeter R. D., Iebringer, R. R., Quaife, C. J., Maxwell, F., Maxwell, I. H., and Brinster, R. L. (1987) Cell 50, 435–443

5 T. Sakurai, T. Moriguchi, and Yamanaka, A., unpublished observation.