Cloning and characterization of the carp prolactin gene

Huang-Tsu Chen¹, Chien-Shun Chiou¹ and Wen-Chang Chang^{1,2}

¹ Institute of Biological Chemistry, Academia Sinica and ² Institute of Biochemical Sciences, National Taiwan University, Taipei (Taiwan, China)

(Received 21 November 1990)

Key words: Prolactin; Genomic sequence; Palindrome; (Carp)

A carp genomic DNA clone containing the carp prolactin (Prl) gene was isolated with carp Prl cDNA as a probe. The organization of the carp *Prl* gene was determined by restriction nuclease mapping and nucleotide sequencing. The *Prl* gene comprises approx. 2.8 kilobasepairs (kb) of DNA including the 5'-flanking region, five exons, four introns and the 3'-flanking region. Analysis of the 5'-flanking region reveals (1) the sequence TATATAAT at positions –38 to –31 upstream from the cap site which was found to be a guanine residue, and (2) the palindrome, CTCATTGCATA-TACAAATGAG at positions –79 to –59. The carp *Prl* gene matches with the reported cDNA except for one difference in coding region and five in the 3'-flanking region, while the encoded amino acid sequences are identical. The arrangement of exons and introns is very similar to that seen in carp GH as well as mammalian *Prl*, which, however, have much longer introns.

Fig. 1. The restriction map of carp *Prl* gene in EMBL 3 (A) and the sequencing strategy for the subcloned fragment in pGEM 4 (B). The arrows show the direction of sequencing by the chain-termination method. P, TATA box; MCS, multiple cloning site; solid box, coding region; open box, untranslated region; lines between the boxes, introns.

The sequence data in this paper have been submitted to the EMBL/Genbank Data Libraries under the accession number X52881. Abbreviations: Prl, prolactin; GH, growth hormone.

Correspondence: W.C. Chang, Institute of Biological Chemistry, Academia Sinica, P.O. Box 23-106, Taipei, Taiwan.

Prolactin (Prl) is a polypeptide hormone produced and secreted by the anterior pituitary gland [1]. It is a member of a family of hormones that includes Prl, growth hormone (GH) and placental lactogen. This family is believed to have evolved from a single ancestral gene by duplication and sequence divergence [2].

Fig. 2. The complete nucleotide sequence of carp *Prl* gene. Exons are shown by capitals and introns by lower case letters. The numbering is given in the right margin and begins at the cap site (+1). Encoded amino acid residues are represented by one-letter codes below the codons. The bases in mRNA [17] are shown below the genomic sequence at positions of discrepancy indicated by asterisks. The upstream palindrome, TATA box and the down stream polyadenylation signal are underlined.

Fig. 2. The complete nucleotide sequence of carp *Prl* gene. Exons are shown by capitals and introns by lower case letters. The numbering is given in the right margin and begins at the cap site (+1). Encoded amino acid residues are represented by one-letter codes below the codons. The bases in mRNA [17] are shown below the genomic sequence at positions of discrepancy indicated by asterisks. The upstream palindrome, TATA box and the down stream polyadenylation signal are underlined.

Prl gene expression is under complex hormonal control. Many studies had shown that thyroid hormone, glucocorticoids, dopamine, etc. [3–7] directly regulate the levels of *Prl* and/or GH mRNA. *Prl* and GH transcription is generally regulated in opposite direction by most of the above-mentioned hormones, providing a very interesting system to study the mechanisms of hormonal control of gene expression.

In vertebrates, *Prl* is involved in many physiological functions, the best known being lactogenesis [8]. In teleosts, the hormone is implicated in osmoregulation, electrolyte balance and many other functions [9,10]. Prunet et al. [11] found that *Prl* expression in rainbow trout might be effected by changes in water salinity. However, there is still little information concerning the role of *Prl* in fish adaptation to new osmotic environments [12,13]. These features make *Prl* an interesting gene to study, especially with respect to the potential control sequence that may be located in the 5'-flanking region of this gene [14,16]. Analysis of the *Prl* gene could provide some insight into the structures which are involved in the gene expression. Furthermore, this may

contribute to an understanding of mechanisms which facilitate the differential expression and regulation of *Prl* gene.

As an integral part of our studies on the structure, function and molecular biology of fish pituitary hormones [17–19], the carp *Prl* gene was cloned, sequenced and characterized. These data will be essential for further studies on the regulation and functions of *Prl* gene in fish.

Eggs collected from common carp (*Cyprinus carpio*) were immediately frozen in liquid nitrogen and stored at -70°C . Genomic DNA was prepared from the carp eggs and partially digested with *Bam*HI. Fragments of 10–20 kb were recovered from 0.5% agarose gel and ligated with λ phage EMBL3 arms. In vitro package and transfection were carried out according to the protocols of the commercial supplier (Promega).

The carp genomic library was screened with ^{32}P -labeled carp *Prl* cDNA [17] as probe. The positive clones were digested with suitable restriction enzymes and subcloned in plasmid pGEM 4. Recombinant phage and plasmid were purified by the standard procedures [20]. DNA sequence was determined by chain-termination method using denatured plasmid templates [21].

The transcription start site was determined by primer extension using carp pituitary mRNA as template. The mRNA was prepared as previously described [19] and annealed to a synthetic primer 5'-GTCTAGATCCT-TGAGTCAT-3' which is complementary to the first six codons of exon 1 in the genomic sequence (Fig. 2). The primer extension was carried out with the mouse Moloney leukemia virus reverse transcriptase. The ^{32}P -labeled reaction products were analyzed by electrophoresis in 10% polyacrylamide sequencing gel.

From a library of $2 \cdot 10^5$ plaques, one clone was found to hybridize strongly with the radiolabeled cDNA probe. Restriction enzyme mapping and hybridization with the cDNA probe revealed that the positive clone contained a *Prl* gene in an insert of about 15 kb (Fig. 1). The exon-containing fragments were identified by further digestion and hybridization and were subcloned in pGEM 4 vector and sequenced (Fig. 1). The complete nucleotide sequence was shown in Fig. 2 together with the encoded amino acid sequence of carp *Prl*. Comparison of the genomic sequence with that of the cDNA reveals identical amino acid sequence of the gene product and only one base-change in the coding region and 5 more in the 3'-untranslated region of the last exon (Fig. 2). Certainly this 2.7 kb DNA fragment contains the full-length *Prl* gene which would give rise to the cDNA previously cloned [17]. A TATA box was found in the 5'-flanking region from -38 to -31 (Fig. 2). Further upstream was a 21 bp palindrome CTCAT-TGCATATACAAATGAG from -59 to -79. The CAAT box was not found in this region. All the intron-exon boundaries followed the GT/AG rule [22].

Fig. 3. Determination of the transcription start site. Lanes G, A, T and C indicate the size markers from dideoxy sequencing reaction products of vector mp18 as template with a suitable primer. Lane P is the primer-extension products generated using synthetic oligonucleotide primer and mRNA. The asterisk indicates the guanine residue at transcription start site.

Fig. 4. Comparison of structures of the *Prl* genes of carp (this study), human [16] and rat [6] and of the carp *GH* gene [18]. Solid boxes, coding regions; open boxes, untranslated regions; lines between the boxes, introns.

The transcription start site was determined by the primer extension method (Fig. 3) and found to be a guanine residue, located 38 bp downstream from the TATA box (Fig. 2).

As shown in Fig. 4, the *Prl* and *GH* [18] genes in carp are very similar in terms of size, number and length of exons and introns. However, they are much shorter as compared to mammalian genes, due to the smaller introns in the carp genes as is the case for carp γ -crystallin genes (unpublished data). The carp *Prl* and *GH* also exhibit 50% homology in their coding sequence, appreciably higher than rat and human, which have around 40% sequence homology in the *Prl* and *GH* coding regions (data not shown).

This study was supported by a grant from the National Science Council of the Republic of China.

References

- Niall, H.D., Hogan, M.L., Sauer, R., Rosenblum, I.Y. and Greenwood, F.C. (1971) *Proc. Natl. Acad. Sci. USA* 68, 866–869.
- Li, C.H. (1980) in *Hormonal Proteins and Peptides* (Li, C.H., ed.), Vol. 8, pp. 1–36, Academic Press, New York.
- Tashjian, A.H., Bancroft, F.C. and Levine, L. (1979) *J. Cell Biol.* 47, 61–70.
- Gourdji, D., Tougaard, C. and Tixier-Vidal, A. (1982) in *Frontiers in Neuroendocrinology* (Ganong, W.F. and Martini, L., eds.), Vol. 7, pp. 317–357, Raven Press, New York.
- Maurer, R.A. (1981) *Nature* 294, 94–97.
- Maurer, R.A., Ermin, C.R. and Donelson, J.E. (1981) *J. Biol. Chem.* 256, 10524–10528.
- Murdoch, G.H., Potter, E., Nicolais, A.K., Evans, R.M. and Rosenfeld, M.G. (1982) *Nature* 300, 192–194.
- Emmerman, J.T., Enami, J., Pitelka, D.R. and Nandi, S. (1977) *Proc. Natl. Acad. Sci. USA* 74, 4466–4470.
- Clarke, W.C. and Bern, H.A. (1980) in *Hormonal Proteins and Peptides*, (Li, C.H., ed.), Vol. 8, pp. 105–197, Academic Press, New York.
- Ensor, D.M. (1978) in *Comparative Endocrinology of Prolactin*, pp. 15–44, Chapman and Hall, London.
- Prunet, P., Boeuf, G. and Houdebine, L.M. (1985) *J. Exp. Zool.* 235, 187–196.
- Loretz, C.A. and Bern, H.A. (1982) *Neuroendocrinology* 35, 292–304.
- Hirano, T. (1986) in *Comparative Endocrinology Developments and Directions* (Ralph, C.L., ed.), Alan R. Liss, New York.
- Gubbins, E.J., Maurer, R.A., Lagrimini, M., Ermin, C.R. and Donelson, J.E. (1980) *J. Biol. Chem.* 255, 8655–8662.
- Camper, S.A., Luck, D.N., Yao, Y., Woychi, R.P., Goodwin, R.G., Lyons, R.H. and Rottman, F.M. (1984) *DNA* 3, 237–249.
- Truong, A.T., Duez, C., Belayew, A., Renard, A., Pictet, R., Bell, G. and Martial, J.A. (1984) *EMBO J.* 3, 429–437.
- Chao, S.C., Pan, F.M. and Chang, W.C. (1988) *Nucleic Acids Res.* 16, 9350–9350.
- Chiou, C.S., Chen, H.T. and Chang, W.C. (1990) *Biochim. Biophys. Acta* 1087, 91–94.
- Chao, S.C., Pan, F.M. and Chang, W.C. (1989) *Biochim. Biophys. Acta* 1007, 233–236.
- Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning. A laboratory manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Hattori, M. and Sakaki, Y. (1986) *Anal. Biochem.* 152, 232–238.
- Breathnach, R. and Chambon, P. (1981) *Annu. Rev. Biochem.* 50, 349–383.