Volume 228, number 2, 301-304 FEB 05577 February 1988

Cloning and sequencing of cDNA that encodes goat growth hormone

Yoshiaki Yamano, Kazuo Oyabayashi, Mitsuhiro Okuno, Miki Yato, Noriyuki Kioka, Eichi Manabe, Hidetaka Hashi, Hiroshi Sakai, Tohru Komano, Kyozo Utsumi* and Akira Iritani*

Laboratory of Biochemistry, Department of Agricultural Chemistry and **Laboratory of Animal Reproduction, Department of Animal Science, Kyoto University, Kyoto 606, Japan*

Received 16 October 1987

The cDNA that encodes goat growth hormone (gGH) was isolated from a goat pituitary cDNA library. The cDNA, about 880 base pairs long, had a coding sequence, 5'- and 3'-untranslated regions and a poly(A) chain. The cDNA could encode a polypeptide of 217 amino acids. The amino acid sequence homology between gGH and the sequences of bovine GH, rat GH and human GH was 99, 83 and 66%, respectively. By Northern blot hybridization, we found that the possible gGH gene is transcribed in the goat pituitary.

Growth hormone; cDNA cloning; Nucleotide sequence; (Goat pituitary)

1. INTRODUCTION

Growth hormone (GH; somatotropin) essential for linear growth in vertebrates has been isolated from the pituitary. GH genes have been isolated from several mammalian species [1–7] and lower vertebrates such as fish [8], and characterized in detail. The primary structure of the GH genes must be compared to study the evolution and regulation of the expression of the genes. The goat is an important domesticated animal, but we have no information about its GH gene structure or amino acid sequence. We cloned a full-length cDNA molecule encoding goat GH (gGH), and report here the nucleotide sequence of the cDNA and the primary structure of the precursor protein molecule that we have deduced. We also performed Northern blot analysis and demonstrated transcription of the gGH gene in the goat pituitary.

Correspondence address: Y. Yamano, Laboratory of Biochemistry, Department of Agricultural Chemistry, Kyoto University, Kyoto 606, Japan

The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00767

2. MATERIALS AND METHODS

Poly(A) RNA was prepared from pituitary of goat (*Capra hircus* L. Saanen) by the guanidinium isothiocyanate method [9], further purified by oligo(dT)-cellulose chromatography, and used for constructing a cDNA library [10,11] in the Okayama-Berg cloning vector pcDV1. About 2×10^4 independent transformants of *Escherichia coli* DH1 were obtained.

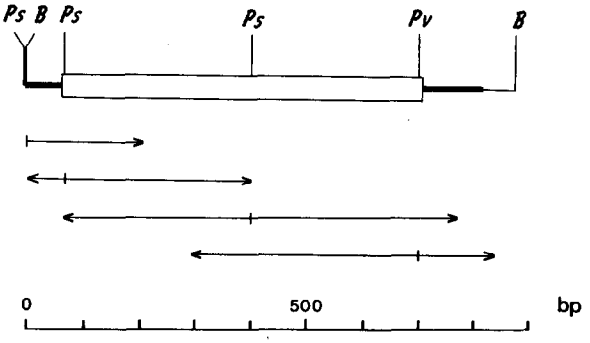

Fig.1. Restriction map of pgcGH-24. From left to right, the diagram shows the G-C tail (thin line), the 5'-untranslated region (thick line), the region coding for gGH (open box), the 3'-untranslated region (thick line), and the A-T tail (thin line). The sequencing strategy is indicated below the map. Restriction enzyme cleavage sites: *Ps*, *Pst*I; *B*, *Bam*HI; *Pv*, *Pvu*II. bp, base pairs.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

```
         10          20          30          40          50
AGGATCCCAG GACCCAGTTC ACCAGACGAC TCAGGGTCCT GCTGACAGCT CACCAACT ATG ATG GCT GCA
                                                                M   M   A   A

 73                  88                 103                 118
GGC CCC CGG ACC TCC CTG CTC CTG GCT TTC ACC CTG CTC TGC CTG CCC TGG ACT CAG GTG
G   P   R   T   S   L   L   L   A   F   T   L   L   C   L   P   W   T   Q   V

133                 148                 163                 178
GTG GGC GCC TTC CCA GCC ATG TCC TTG TCC GGC CTG TTT GCC AAC GCT GTG CTC CGG GCT
V   G   A   F   P   A   M   S   L   S   G   L   F   A   N   A   V   L   R   A

193                 208                 223                 238
CAG CAC CTG CAT CAA CTG GCT GCT GAC ACC TTC AAA GAG TTT GAG CGC ACC TAC ATC CCG
Q   H   L   H   Q   L   A   A   D   T   F   K   E   F   E   R   T   Y   I   P

253                 268                 283                 298
GAG GGA CAG AGA TAC TCC ATC CAG AAC ACC CAG GTT GCC TTC TGC TTC TCT GAA ACC ATC
E   G   Q   R   Y   S   I   Q   N   T   Q   V   A   F   C   F   S   E   T   I

313                 328                 343                 358
CCG GCC CCC ACG GGC AAG AAT GAG GCC CAG CAG AAA TCA GAC TTG GAG CTG CTT CGC ATC
P   A   P   T   G   K   N   E   A   Q   Q   K   S   D   L   E   L   L   R   I

373                 388                 403                 418
TCA CTG CTC CTT ATC CAG TCG TGG CTT GGG CCC CTG CAG TTC CTC AGC AGA GTC TTC ACC
S   L   L   L   I   Q   S   W   L   G   P   L   Q   F   L   S   R   V   F   T

433                 448                 463                 478
AAC AGC CTG GTG TTT GGC ACC TCG GAC CGT GTC TAT GAG AAG CTG AAG GAC CTG GAG GAA
N   S   L   V   F   G   T   S   D   R   V   Y   E   K   L   K   D   L   E   E

493                 508                 523                 538
GGC ATC CTG GCC CTG ATG CGG GAG CTG GAA GAT GTT ACC CCC CGG GCT GGG CAG ATC CTC
G   I   L   A   L   M   R   E   L   E   D   V   T   P   R   A   G   Q   I   L

553                 568                 583                 598
AAG CAG ACC TAT GAC AAA TTT GAC ACA AAC ATG CGC AGT GAC GAC GCG CTG CTC AAG AAC
K   Q   T   Y   D   K   F   D   T   N   M   R   S   D   D   A   L   L   K   N

613                 628                 643                 658
TAC GGT CTG CTC TCC TGC TTC CGG AAG GAC CTG CAC AAG ACG GAG ACG TAC CTG AGG GTC
Y   G   L   L   S   C   F   R   K   D   L   H   K   T   E   T   Y   L   R   V

673                 688                 703                 722         732
ATG AAC TGT CGC CGC TTC GGG GAG GCC AGC TGT GCC TTC TAG TTGCCAGCCA TCTGTTGTTA
M   K   C   R   R   F   G   E   A   S   C   A   F   *

        742         752         762         772         782         792         802
CCCCTCCCCG TGCCTTCCTA GACCCTGGAA GGTGCCACTC CAGTGCCCAC TGTCCTTTCC TAATAAAGCG

        812
AGGAAATTGC ATCAC··(Poly A)····
```

Fig.2. Nucleotide sequence of gGH cDNA and the amino acid sequence predicted. Numbering of the nucleotide sequence in the 5′ to 3′ direction begins at the first nucleotide of the cDNA. TAG (position 710 to 712) is the termination triplet. The poly(A) addition signal is underlined.

Colonies containing gGH sequences were identified by colony hybridization [12]. Bovine-GH cDNA (pG23) was used as a probe in the hybridization experiment [1]. The gGH cDNA was isolated from one positive clone. The nucleotide sequence was determined by the chain-termination method [13,14] with use of restriction fragments of cDNA subcloned in the phage M13mp18 and mp19. Northern blot analysis was done by the method of Thomas [15] with nitrocellulose membrane and purified poly(A) RNA.

3. RESULTS AND DISCUSSION

A cDNA library constructed from goat pituitary mRNA in the cloning vector, pcDV1, was screened for gGH cDNA. Out of 2×10^4 colonies, 40 colonies contained cDNA clones hybridizable to a bovine cDNA used as a probe. One of them, designated pgcGH-24, carried the longest cDNA

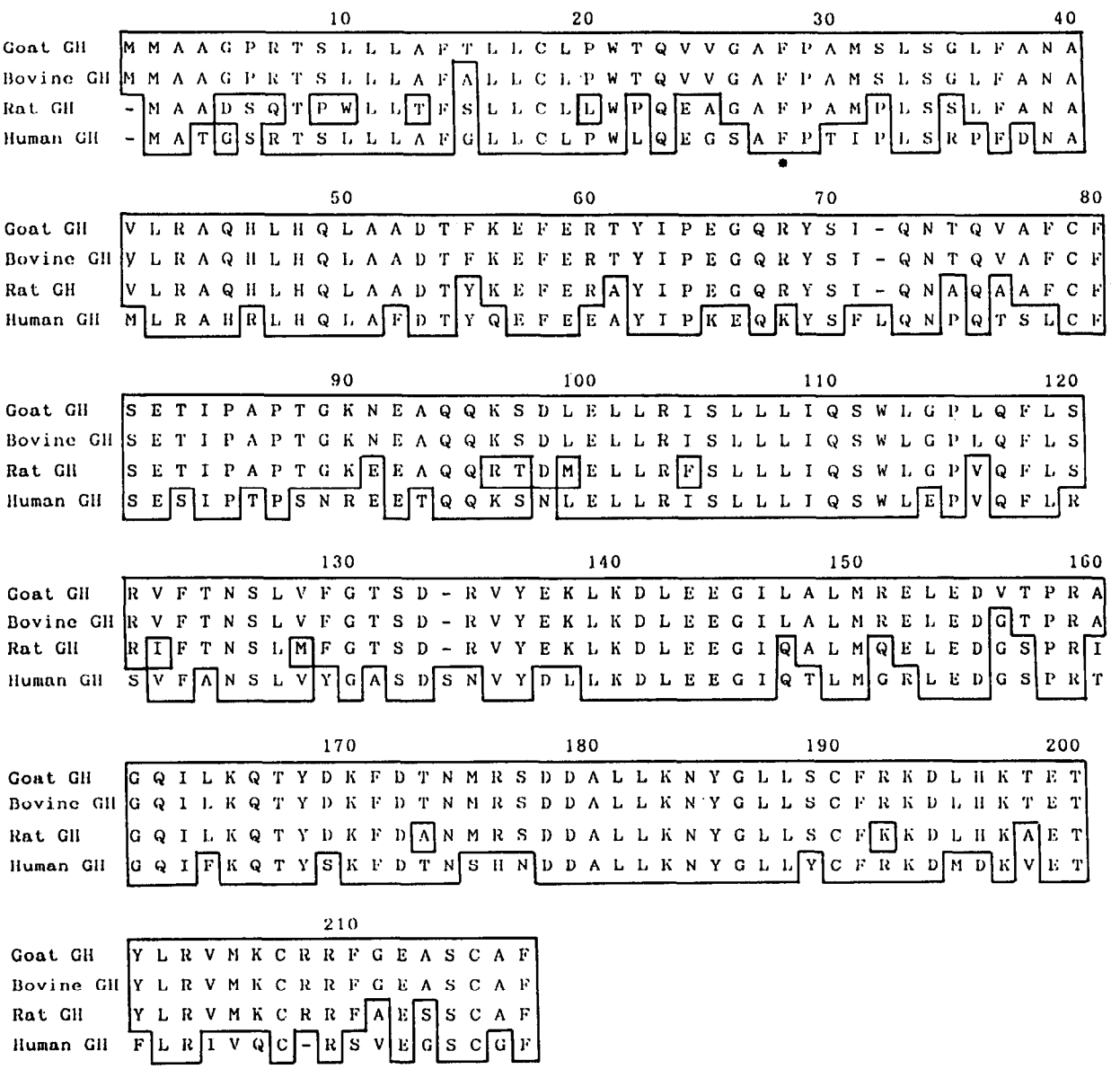

Fig.3. Comparison of amino acid sequences of goat GH (gGH), bovine GH, rat GH and human GH. The amino acid sequences were aligned by the introduction of gaps to maximize homology. Amino acid residues identical to gGH are boxed. The NH_2-terminal amino acids of mature hormone (F for bovine GH, rat GH, and human GH) are shown by an asterisk.

tract of about 880 base pairs. The restriction map of the cDNA and the DNA sequencing strategy are shown in fig.1. The nucleotide sequence of the gGH cDNA and the amino acid sequence that we deduced for gGH are shown in fig.2. Clone pgcGH-24 contained 58 bases of a 5′-noncoding sequence followed by an open reading frame of 651 bases encoding a polypeptide of 217 amino acids (including a signal sequence). The 3′-untranslated region of 105 bases contained the hexanucleotide AATAAA, which precedes the polyadenylation site. This is a structure common to many other eukaryotic mRNAs [16]. The poly(A) tract is 60 bases long. Homology in amino acid sequences between gGH and bovine GH [1], rat GH [4] and human GH [6,7] was 99, 83 and 66%, respectively (fig.3). The amino acid sequence of bovine GH, and that of gGH differ only in two amino acids, positions 15 (Ala changed to Thr in the goat) and 156 (Gly changed to Val). The homology in the amino acid sequences may be attributed to goats and bovine being closely related to each other in evolution. The 27 NH_2-terminal amino acids encoded by the cDNA may be a signal peptide, to judge from the GH sequences of other animals. With bovine GH cDNA as a probe, we performed Northern hybridization of poly(A) RNA from goat pituitary. An mRNA 880 bases long was hybridizable to the probe (fig.4). The results showed that the potential gGH gene is transcribed in the goat pituitary.

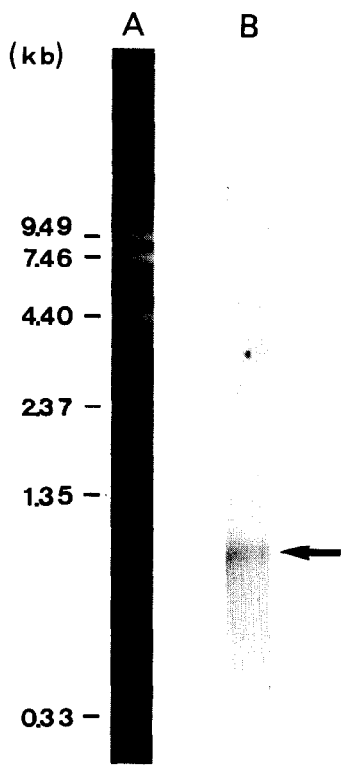

Fig.4. Northern blot hybridization of gGH mRNA. The size markers were provided by Bethesda Research Laboratories (RNA ladders). The markers were treated with formaldehyde, as was the poly(A) RNA (A). Poly(A) RNA (1 μg) isolated from goat pituitary was hybridized with bovine GH cDNA probe pG23. The hybridization position is indicated by the arrow (B).

Acknowledgements: We thank Dr K. Shigesada for suggestions about cDNA cloning and Dr F.M. Rottman for providing us with bovine growth hormone gene. This work was supported in part by a scientific grant from the Ministry of Education, Science and Culture, Japan, and by the Takeda Science Foundation, Japan.

REFERENCES

[1] Woychik, R.P., Camper, S.A., Lyons, R.H., Horowitz, S., Goodwin, E.C. and Rottman, F.M. (1982) Nucleic Acids Res. 10, 7197–7210.
[2] Gordon, D.F., Quick, D.P., Erwin, C.R., Donelson, J.E. and Maurer, R.A. (1983) Mol. Cell. Endocrinol. 33, 81–95.
[3] Seeburg, P.H., Shine, J., Martial, J.A., Baxter, J.D. and Goodman, H.M. (1977) Nature 270, 486–494.
[4] Page, G.S., Smith, S. and Goodman, H.M. (1981) Nucleic Acids Res. 9, 2087–2104.
[5] Martial, J.A., Halliwell, R.A., Baxter, J.D. and Goodman, H.M. (1979) Science 205, 602–607.
[6] Roskam, W.G. and Rougeon, F. (1979) Nucleic Acids Res. 7, 305–320.
[7] DeNoto, F.M., Moore, D.D. and Goodman, H.M. (1981) Nucleic Acids Res. 9, 3719–3730.
[8] Sekine, S., Mizukami, T., Nishi, T., Kuwana, Y., Saito, A., Sato, M., Itoh, S. and Kawauchi, H. (1985) Proc. Natl. Acad. Sci. USA 82, 4306–4310.
[9] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[10] Okayama, H. and Berg, P. (1982) Mol. Cell. Biol. 2, 161–170.
[11] Okayama, H. and Berg, P. (1983) Mol. Cell. Biol. 3, 280–289.
[12] Southern, E.M. (1975) J. Mol. Biol. 98, 503–517.
[13] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[14] Messing, J. and Vieira, J. (1982) Gene 19, 269–276.
[15] Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201–5205.
[16] Proudfoot, N.J. and Brownlee, G.G. (1976) Nature 263, 211–214.