Molecular cloning and nucleotide sequence analysis of complementary DNA for bullfrog prolactin

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ABSTRACT

A prolactin cDNA was cloned from a cDNA expression library constructed from total RNA of bullfrog (*Rana catesbeiana*) adenohypophyses by immunoscreening with antiserum against bullfrog prolactin. The cDNA clone thus obtained contained a 249 bp insert. Using this clone as a probe, plaque hybridizations were performed and two additional clones obtained. These clones had a polyadenylation site different from that of the first obtained clone, suggesting that the 3′-untranslated sequence was heterogeneous in length. The longest clone contained 830 bp, which encoded part of the signal peptide and the entire sequence of mature prolactin. The deduced amino acid sequence was in good accord with that determined by direct protein sequencing of purified bullfrog prolactin. The length of the bullfrog prolactin mRNA was estimated by Northern blot analysis to be about 1·0 kb. Homologies of prolactin nucleotide and amino acid sequences between bullfrog and other vertebrates were 64 and 65% for man, 66 and 68% for pig, 61 and 52% for rat, 69 and 74% for chicken, and 50 and 35% for salmon respectively. Highly conserved regions reported for mammalian prolactins also existed in bullfrog prolactin. Homologies of nucleotide and amino acid sequences between prolactin and GH of bullfrog origin were 49 and 25% respectively. Using the cDNA, the content of prolactin mRNA in the pituitary glands of metamorphosing tadpoles was measured. Prolactin mRNA levels rose at the mid-climax stage, suggesting that the increase in plasma and pituitary prolactin levels known to occur at the climax stage accompanies the increase in prolactin synthesis.

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INTRODUCTION

Prolactin is known to have versatile biological actions in vertebrates. In amphibians, it is involved in growth, metamorphosis, osmoregulation and reproduction (Nicoll & Bern, 1972; Ensor, 1978; Kikuyama, Yamamoto & Seki, 1980; Bern, 1983). However, most of the information about the biological actions of prolactin in amphibians has been obtained from experiments in which mammalian prolactins were used exclusively. Purified amphibian prolactins became available comparatively recently (Yamamoto & Kikuyama, 1981; Yamamoto, Kobayashi & Kikuyama, 1986*a*; Matsuda, Yamamoto & Kikuyama, 1990) and therefore there have been very few studies of a molecular nature on amphibian prolactin. We have recently purified bullfrog prolactin and subjected it to direct protein sequencing (Kawauchi & Yasuda, 1988; Yasuda, Yamaguchi, Kobayashi *et al.* 1990). This revealed that bullfrog prolactin shows closer similarity to prolactins of other tetrapods than to teleost prolactins. In the present experiments, attempts were made to obtain a bullfrog prolactin cDNA clone in order to analyse its nucleotide sequence, to characterize the mRNA and to apply the cDNA to measurement of prolactin mRNA levels in the pituitary gland of bullfrogs. A preliminary report of this work has appeared elsewhere (Yoshihama, Kikuyama, Yamamoto *et al.* 1989).

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MATERIALS AND METHODS

Construction of a cDNA library and screening of bullfrog prolactin cDNA

Total RNA (about 600 μg) was extracted from 100 adenohypophyses of the bullfrog (*Rana catesbeiana*) using guanidium isothiocyanate, and subjected to density-gradient centrifugation through a caesium chloride cushion according to the method described by Chirgwin, Przybyla, MacDonald & Rutter (1979) using an RNA extraction kit (Amersham International plc, Amersham, Bucks, U.K.). The double-stranded cDNA was synthesized according to the method of Gubler & Hoffmann (1983) and ligated to the EcoRI site of a vector, λ ZAP (Stratagene, La Jolla, CA, U.S.A.). An original library of $2{\cdot}0 \times 10^5$ independent clones was constructed and amplified.

Immunoscreening was performed from 2×10^5 plaques according to the method of Young & Davis (1983). Fusion proteins were expressed on nitrocellulose filters previously soaked in 10 mM isopropyl β-D-thiogalactopyranoside overnight at 37 °C. Plaques reacting with a polyclonal antiserum (1:1000) against the bullfrog prolactin were detected with ^{125}I-labelled protein A prepared by the chloramine-T method as described by Hunter & Greenwood (1962). Absence of cross-reaction of the antiserum used here with other related hormones, such as bullfrog growth hormone (GH), has been established by radioimmunoassay, immunodiffusion test (Yamamoto & Kikuyama, 1982), immunohistochemistry (Kobayashi & Kikuyama, 1990) and immunoblot analysis.

Plaque hybridization was performed at 50 °C in a hybridization solution consisting of 6 × SSC (1 × SSC is 0·15 M NaCl and 0·015 M sodium citrate; pH 7·5), 0·02% (w/v) bovine serum albumin, 0·02% (w/v) ficoll, 0·02% (w/v) polyvinylpyrrolidone and 1% (w/v) sodium dodecylsulphate (SDS). Transfer of the subclone into a plasmid vector was subsequently performed by auto-excision of the Bluescript vector SK M13$^+$ (Stratagene) from the phage vector using a helper phage (R408) according to the instruction manual provided by the supplier. The nucleotide sequences of the cDNAs were determined by a chain-termination method (Sanger, Nicklen & Coulson, 1977) using [α-^{32}P]dCTP for the alkaline-denatured double-stranded DNA (Hattori & Sakaki, 1986) or a fluorescence-labelled primer (Connell, Fung, Heiner *et al.* 1987) with Sequenase (United States Biochemical, Cleveland, OH, U.S.A). The fluorescence-labelled nucleotides were analysed on an Applied Biosystems 370A automated DNA sequencer (Foster City, CA, U.S.A.).

Blot analysis of bullfrog prolactin mRNA

Prolactin mRNA concentrations were assessed by Northern blot analysis (Lehrach, Diamond, Wozney & Boedtker, 1977) and dot-blot hybridization (White & Bancroft, 1982). Total RNA, prepared as described above, was denatured with formaldehyde, electrophoresed on a denaturing gel containing 1% agarose plus 2·2 M formaldehyde and transferred to a Zeta-Probe nylon membrane (Bio-Rad, Richmond, CA, U.S.A.). The filter was irradiated with u.v. light at 302 nm (UVP, San Gabriel, CA, U.S.A.) for 15 min to fix the RNA, under conditions described previously (Kato & Hirai, 1989). Double-stranded cDNA was labelled by the random priming method (Feinberg & Vogelstein, 1983) using an oligolabelling kit (Amersham International plc).

Dot-blot analysis was performed for RNAs from the anterior pituitary glands of bullfrog tadpoles at stages 18–25 (Taylor & Korllos, 1946). Tissues were removed from the tadpoles and stored at −80 °C until extraction of the RNA. Each sample, consisting of five pituitaries (about 500 μg wet weight) from tadpoles at the same developmental stage, was homogenized in guanidium thiocyanate solution with addition of 1 μg *Escherichia coli* tRNA to improve the recovery of the pituitary RNA using an RNA extraction kit (Amersham International plc). Total RNAs, denatured as described above, were applied to a nylon membrane using a Bio-Dot apparatus (Bio-Rad) followed by fixation by u.v. irradiation.

After boiling in 1 × SSC for 3 min, the filters were soaked for 3 h in a hybridization solution described above. Hybridization was performed in the solution containing labelled cDNA overnight at 68 °C. The filters were washed twice in 0·1 × SSC containing 1% SDS for 15 min each at 42 °C and exposed to X-ray film with an intensifying screen (Toshiba E-32) for 1 or 3 days at −80 °C.

Densitometry was performed on the dot-blot autographs with a Joyce-Loeble Chromoscan 3 densitometer. After removing the label by boiling in 1 × SSC for 3 min, rehybridization was achieved with chicken β-actin cDNA (Oncor Inc., Gaithersburg, MD, U.S.A.). For normalization, the densitometry values for the prolactin mRNA were divided by those for the β-actin mRNA. Statistical analysis was performed by Duncan's new multiple range test.

RESULTS

Isolation and nucleotide sequence of bullfrog prolactin cDNA

An immunoreactive clone designated pfPRL-4 was obtained by immunoscreening of a cDNA library of

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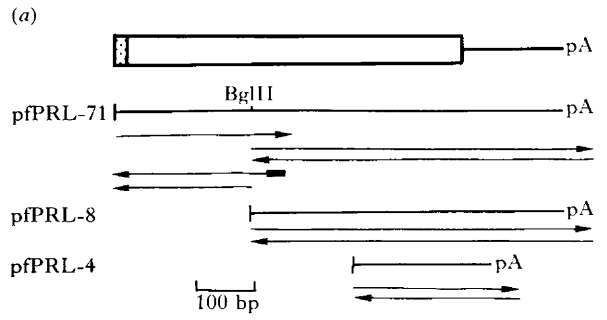

(b)

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TTA. CTA. GCA. GGA. ATT. GGG. GTG. ACA. TCA. CAA. CCA. ATA. TGC. CCA. AAT. GGG. GGC. ACA. AAC.
Leu-Leu-Ala-Gly-Ile-Gly-Val-Thr-Ser-Gln-Pro-Ile-Cys-Pro-Asn-Gly-Gly-Thr-Asn-
 -9              -5              +1                                          +10

TGT. CAA. ATA. CCC. ACG. TCT. GCT. CTC. TTT. GAC. CGT. GCC. GTA. AAA. CTT. TCA. CAT. TAT. ATC. CAT.
Cys-Gln-Ile-Pro-Thr-Ser-Ala-Leu-Phe-Asp-Arg-Ala-Val-Lys-Leu-Ser-His-Tyr-Ile-His-
                                +20                                         +30

TCT. CTT. TCC. TCT. GAA. ATG. TTT. AAT. GAG. TTT. GAT. GAG. CGC. TTT. ACT. CCA. GGA. CGT. AGG. TTT.
Ser-Leu-Ser-Ser-Glu-Met-Phe-Asn-Glu-Phe-Asp-Glu-Arg-Phe-Thr-Pro-Gly-Arg-Arg-Phe-
                                +40                                         +50

CTA. GCA. AAA. TCT. GGA. ATA. AGT. TGC. CAT. ACT. TCT. TCT. CTG. AAT. ACT. CCA. GAA. GAC. AAA. GAG.
Leu-Ala-Lys-Ser-Gly-Ile-Ser-Cys-His-Thr-Ser-Ser-Leu-Asn-Thr-Pro-Glu-Asp-Lys-Glu-
                                +60                                         +70

CAA. GCC. CGA. CAG. ATA. CAA. CAT. GAA. GAT. CTG. CTG. AAC. TTG. GTG. CTG. AAG. GTT. CTA. AGA. TCA.
Gln-Ala-Arg-Gln-Ile-Gln-His-Glu-Asp-Leu-Leu-Asn-Leu-Val-Leu-Lys-Val-Leu-Arg-Ser-
                                +80                                         +90

TGG. AAT. GAT. CCT. TTG. GTT. CAC. ATG. GTT. TCT. GAA. GTG. CAA. GAT. ATC. CGG. GAG. GCT. CCA. GAC.
Trp-Asn-Asp-Pro-Leu-Val-His-Met-Val-Ser-Glu-Val-Gln-Asp-Ile-Arg-Glu-Ala-Pro-Asp-
                                +100                                        +110

ACC. ATC. CTT. TGG. AAA. ACT. GTA. GAG. GTT. GAA. GAG. CAA. ACT. AAA. CGT. CTC. CTC. GAA. GGA. ATG.
Thr-Ile-Leu-Trp-Lys-Thr-Val-Glu-Val-Glu-Glu-Gln-Thr-Lys-Arg-Leu-Leu-Glu-Gly-Met-
                                +120                                        +130

GAA. AGG. ATT. ATT. GGC. AGG. ATT. CAA. CCA. GGT. GAT. TTA. GAA. AAT. GAA. ATT. TAC. TCG. CCA. TGG.
Glu-Arg-Ile-Ile-Gly-Arg-Ile-Gln-Pro-Gly-Asp-Leu-Glu-Asn-Glu-Ile-Tyr-Ser-Pro-Trp-
                                +140                                        +150

CCT. GGC. CCA. GCT. TCA. ATA. CCA. GGA. GAT. GAA. AAT. TCA. CGC. CTC. TTT. GCA. TTC. TAC. AAT. CTA.
Pro-Gly-Pro-Ala-Ser-Ile-Pro-Gly-Asp-Glu-Asn-Ser-Arg-Leu-Phe-Ala-Phe-Tyr-Asn-Leu-
                                +160                                        +170

CTC. CAC. TGC. CTT. CGT. AGG. GAC. TCT. CAC. AAA. ATC. GAC. AAC. TAT. TTG. AAG. CTT. CTA. AAA. TGC.
Leu-His-Cys-Leu-Arg-Arg-Asp-Ser-His-Lys-Ile-Asp-Asn-Tyr-Leu-Lys-Leu-Leu-Lys-Cys-
                                +180                                        +190

CGT. CTT. ATC. CAT. GAG. GGA. AAT. TGT. TGA. ACTTTTATTCTTAACTCTCTACAGCAATAAAGATGTGTTTCTCAA
Arg-Leu-Ile-His-Glu-Gly-Asn-Cys
                                +198

pA
ACAGCAATGATATGAATAAGTCTTTTAGAAAAACATGTGACAAAATCGTTTTTTAATAGTTATATAGAGTTTCTAATAAAT

TGGATTCCTATTTGTAGAATAAAGCCAAATCAAATAAAATGCAAAAATATATGTACATAC(A)n
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FIGURE 1. Sequencing strategy and the nucleotide sequence of bullfrog prolactin cDNA. (*a*) Sequence strategies of three clones (pfPRL-71, -8 and -4) are shown. The rectangle and bold line indicate the coding region and the 3′-untranslated region respectively. The signal peptide is indicated by the stippled rectangle. The direction and length of sequencing are represented by arrows. An oligonucleotide site specific for bullfrog prolactin (small solid rectangle) was synthesized and used as a sequencing primer. Restriction enzyme BglII was used to construct subclones of pfPRL-71, which were sequenced as indicated. Poly(A) tails are indicated by pA. (*b*) The nucleotide sequence of pfPRL-71 is shown. The positions of the 5′ terminus of other clones and the poly(A) tail of pfPRL-4 clones are indicated by arrowheads (above the sequence) and pA respectively. The termination signal TGA is overlined. Polyadenylation signals (AATAAA) are indicated by underlining. The predicted amino acids are shown below the nucleotide sequence. The N-terminal amino acid of the mature protein is numbered +1. The minus numbers indicate the amino acids comprising part of the signal sequence.

bullfrog anterior pituitaries. The clone, consisting of 249 bases, coded the carboxyl part of the prolactin molecule. Independent plaque hybridizations were performed several times using the pfPRL-4 cDNA insert as a probe, and two further clones were obtained. The strategy used and the nucleotide sequence obtained are shown in Fig. 1. The clone having the longest insert, pfPRL-71, contained four polyadenylation signals. The other clone, pfPRL-8, had a nucleotide sequence identical with that of the 3′-untranslated region of pfPRL-71. pfPRL-4 contained a single polyadenylation signal, and its poly(A) tail was at a position different from those of the other two clones. The deduced amino acid sequence (indicated below the nucleotide sequence) showed that pfPRL-71 contained only a partial signal peptide sequence of nine amino acids, but encoded the entire sequence of the mature prolactin molecule consisting of 198 amino acids. This result is consistent with the data for direct protein sequencing of purified bullfrog prolactin obtained previously (Kawauchi & Yasuda, 1988; Yasuda *et al.* 1990) except that the tryptophan residue at position 114 was lacking in the latter. The total sequence of 198 amino acids of the mature molecule, including six cysteine residues, resembled that of other tetrapod prolactins.

Northern blot analysis of prolactin mRNA

Northern blot analysis of prolactin mRNA was performed using a ^{32}P-labelled pfPRL-71 insert, as shown in Fig. 2. A single band showing positive hybridization was detected at a position corresponding to about 1·0 kb.

Hybridization experiments with adenohypophysial RNA from bullfrog tadpoles

The content of prolactin mRNA in the adenohypophyses of metamorphosing tadpoles was measured. There was a linear relationship between the amount of dotted RNA and the density of the dots (data not shown). The content of prolactin mRNA was

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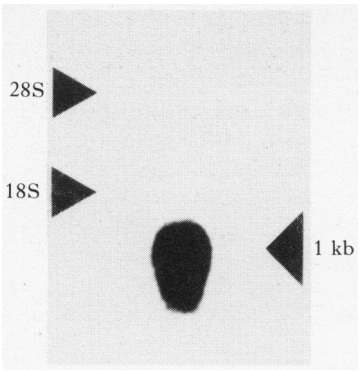

FIGURE 2. Northern blot analysis of bullfrog prolactin. Total RNA prepared from bullfrog anterior pituitaries was analysed on a 1% agarose gel containing 50% formamide and 2·2 M formaldehyde. The positions of ribosomal RNAs (28S and 18S) and 1 kb are indicated.

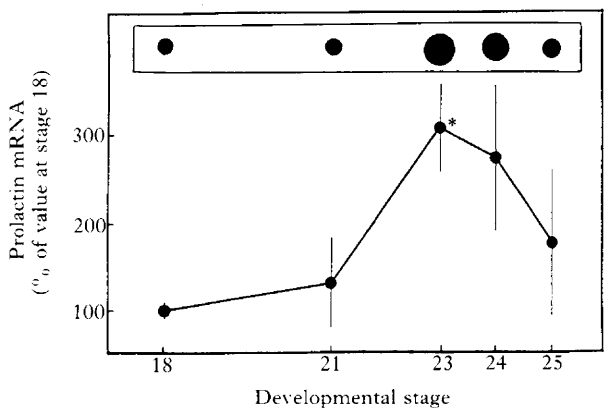

FIGURE 3. Developmental changes in prolactin mRNA in the pituitary glands of metamorphosing tadpoles. Total RNA from the pituitaries of bullfrog tadpoles (stages 18–25) was subjected to dot-blot analysis of prolactin mRNA. Each sample was prepared from five pituitaries. After hybridization with bullfrog prolactin cDNA, the probe was removed and the filter rehybridized with ^{32}P-labelled chick actin cDNA. The autographies are shown in the inset. The densitometry values for prolactin mRNA were divided by those for β-actin mRNA. Prolactin mRNA was expressed as a percentage of the value for animals at stage 18. Values are means ± S.E.M. of four determinations. *$P < 0{\cdot}01$ compared with stage 18 (Duncan's new multiple range test).

divided by that of actin mRNA and expressed as a percentage of the value for animals at stage 18 (Fig. 3). The prolactin mRNA increased as metamorphosis progressed and reached a maximum at stage 23, when it was three times the value at stage 18. The level of prolactin mRNA declined at the end of metamorphosis (stage 25).

DISCUSSION

The amino acid sequence deduced from the cloned prolactin cDNA is in good accord with that obtained by direct protein sequencing of purified bullfrog prolactin (Kawauchi & Yasuda, 1988; Yasuda *et al.* 1990). The sequencing results indicate the presence of a disulphide bridge between residues 4 and 11 at the N-terminal region of the bullfrog prolactin molecule. This disulphide bridge is known to be lacking in teleost prolactins (Yasuda, Itoh & Kawauchi, 1986; Yasuda, Miyajima, Kawauchi *et al.* 1987; Song, Trinh, Hew *et al.* 1988; Yamaguchi, Specker, King *et al.* 1988). It could therefore be said that the presence of a disulphide bridge in the N-terminal region is one of the characteristics of tetrapod prolactins. When the nucleotide and amino acid sequences of bullfrog prolactin are compared with those of other vertebrate prolactins, the homologies are as follows: 64 and 65% for man, 66 and 68% for pig, 61 and 52% for rat, 69 and 74% for chicken, and 50 and 35% for salmon respectively. Interestingly, bullfrog prolactin is closer to bird and mammalian prolactins than the fish prolactin. From the results of Harr plot analysis (Fig. 4), it is evident that the sequence homology between the bullfrog and pig is high compared with that between the bullfrog and salmon. When the nucleotide and amino acid sequences of bullfrog prolactin are compared with those of bullfrog GH (Pan & Chang, 1988), the homologies are 49 and 25% respectively. We have previously described two highly conserved regions common among vertebrate prolactins for which the nucleotide sequences are known (Kato, 1988; Kato, Hirai & Kato, 1990). The nucleotide sequence of bullfrog prolactin also has similar conserved regions (Fig. 5). Several regions of divergence may reflect class- or subclass-specific biological functions. However, investigations on the structure-function relationships of prolactin have so far been inadequate. In this respect, site-directed mutagenesis using prolactin cDNAs would be a useful tool for detecting the loci related to certain biological activities. It is of interest to note that one of the bullfrog prolactin cDNAs is different from the other two with regard to polyadenylation site and length. The mRNA, however, seems to be of a uniform size, judging from the results of Northern blot analysis of prolactin mRNA.

Prolactin is known to have larval growth-promoting activity and anti-metamorphic activity in amphibians (Bern, 1983; Kikuyama & Yamamoto, 1988). Accordingly it has been hypothesized that prolactin levels are high during the preclimax period in order to enhance larval growth, and that they then decline at metamorphic climax to facilitate thyroid

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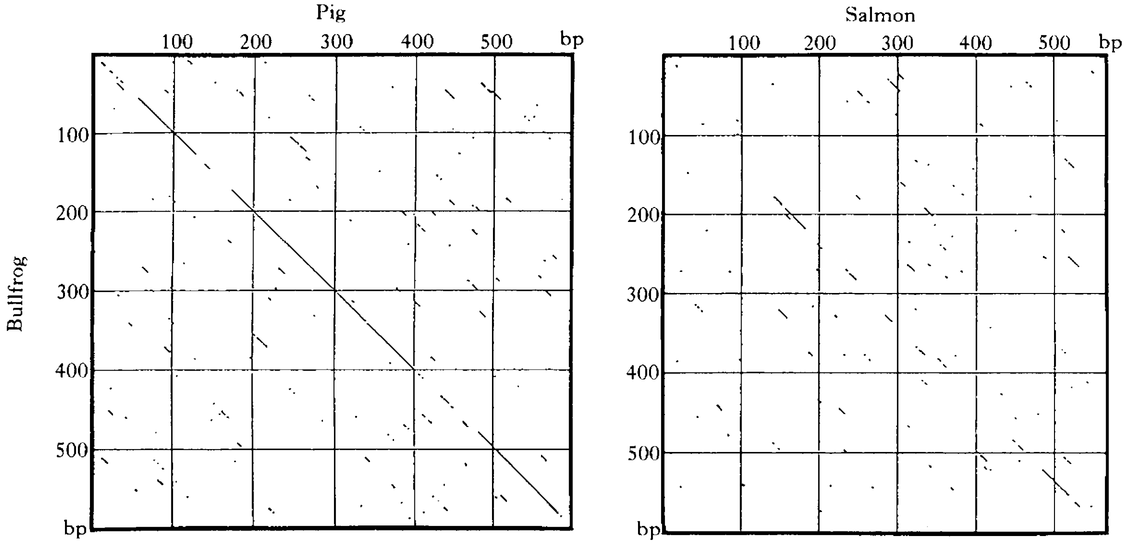

FIGURE 4. Harr plot analysis of the nucleotide sequences of prolactins. Homologies of the nucleotide sequence of bullfrog prolactin with those of porcine and salmon prolactin are shown. Each dot represents a position where 12 nucleotides out of 20 match in both sequences.

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                                                                                           ———— I ————
Human    : LPICPGGAARCQVTLRDLFDRAVVLSHYIHNLSSEMFSEFDKRYTHGRGFITKAINSCHTSSLATPEDKEQAQQM
Porcine  : LPICPSGAVNCQVSLRDLFDRAVILSHYIHNLSSEMFNEFDKRYAQGRGFITKAINSCHTSSLSTPEDKEQAQQI
Bovine   : TPVCPNGPGNRQVSLRDLFDRAVMVSHYIHDLSSEMFNEFDKRYAQGKGFITMALNSCHTSSLPTPEDKEQAQQT
Rat      : LPVCSGG   DCQTPLPELFDRVVMLSHYIHTLYTDMFIEFDKQYVQDREFIAKAINDCPTSSLATPEDKEQAQKV
Mouse    : LPICSAG   DCQTSLRELFDRVVILSHYIHTLYTDMFIEFDKQYVQDREFMVKVINDCPTSSLATPEDKEQALKV
Chicken  : LPICPIGSVNCQVSLGELFDRAVKLSHYIHYLSSEIFNEFDERYAQGRGFITKAVNGCHTSSLTTPEDKEQAQQI
Salmon   :               IGLSDLMERASQRSDKLHSLSTSLTKDLDSHFPPMGRVMMPRPSMCHTSSLQTPKDKEQALKV
Bullfrog: QPICPNGGTNCQIPTSALFDRAVKLSHYIHSLSSEMFNEFDERFTPGRRFLAKSGISCHTSSLNTPEDKEQARQI
                                                         50

Human    : NQKDFLSLIVSILRSWNEPLYHLVTEVRGMQEAPEAILSKAVEIEEQTKRLLEGMEL IVSQVHPETKENEIYPVW
Porcine  : HHEVLLNLILRVLRSWNDPLYHLVTEVRGMQEAPDAILSRAIEIEEQNKRLLEGMEK IVGQVHPGIKENEVYSVW
Bovine   : HHEVVMSLILGLLRSWNDPLYHLVTEVRGMKGAPDAILSRAIEIEEENKRLLEGMEM IFGQVIPGAKETEPYPVW
Rat      : PPEVLLNLILSLVHSWNDPLFQLITGLGGIHEAPDAIISRAKEIEEQNKRLLEGIEK IISQAYPEAKGNEIYLVW
Mouse    : PPEVLLNLILSLVQSSSDPLFQLITGVGGIQEAPEYILSRAKEIEEQNKQLLEGVEK IISQAYPEAKGNGIYFVW
Chicken  : HHEDLLNLVVGVLRSWNDPLIHLASEVQRIKEAPDTILWKAVEIEEQNKRLLEGMEK IVGRVHSGDAGNEIYSHW
Salmon   : SENELISLARYLLLAWNDPLLLLSSEAPTLPH PSNGDISSKIRELQDYSKSLGDGLDIMVNKMGPSSQYISSIPF
Bullfrog: QHEDLLNLVLKVLRSWNDPLVHMVSEVQDIREAPDTILWKTVEVEEQTKRLLEGMER IIGRIQPGDLENEIYSPW
                             100                                                    150
                                          ———— II ————
Human    · SGLPSLHMADEESRLSAYYNLLHCLRRDSHKIDNYLKLLKCRI   IHNNNC
Porcine  : SGLPSLQMADEDTRLFAFFNLLHCLRRDSHKIDNYLKLLKCRI·  IYDSNC
Bovine   : SGLPSLQTKDEDARYSAFYNLLHCLRRDSSKIDTYLKLLNCRI   IYNNNC
Rat      : SQLPSLQGVDEESKDLAFYNNIRCLRRDSHKVDNYLKFLRCQI   VHKNNC
Mouse    : SQLPSLQGVDEESKILSLRNTIRCLRRDSHKVDNFLKVLRCQI   AHQNNC
Chicken  : DGLPSLQLADEDSRLFAFYNLLHCLRRDSHKIDNYLKVLKCRL   IHDSNC
Salmon   : KGGD LGN DKTSRLINFHFLMSCFRRDSHKIDSFLKVLRCRATNMRPETC
Bullfrog: PGPASIPG DENSRLFAFYNLLHCLRRDSHKIDNYLKLLKCRL   IHEGNC
                                                    198
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FIGURE 5. Homology of predicted amino acid sequences of various prolactins. The sequences were predicted by molecular cloning. Amino acids (one letter) conserved throughout the species compared are shown by white letters. Two regions highly conserved in tetrapod prolactins (I and II) are indicated by overlining. Gaps have been introduced to obtain maximal homology. Sequences for man (Cooke, Coit, Shine *et al.* 1981), pig (Kato *et al.* 1990), ox (Sasavage, Nilson, Holowitz & Rottman, 1982), rat (Cooke, Coit, Weiner *et al.* 1980), mouse (Linzer & Talamantes, 1985), chicken (Hanks, Alonzi, Sharp & Sang, 1989), salmon (Song, Trinh, Hew *et al.* 1988) and bullfrog (present study) are shown.

hormone-induced metamorphosis. It was found previously that prolactin levels are relatively low during the preclimax period and do not fall at the onset of climax, but rise during late climax (Yamamoto & Kikuyama, 1982). The results obtained in the present experiment indicate that tissue mRNA levels rise at mid-climax (stage 23), the increase slightly preceding that of the plasma levels of

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prolactin. This suggests that prolactin synthesis is stimulated at mid-climax. In fact, the prolactin content also increases during climax and reaches a maximum at stage 24 (Yamamoto, Niinuma & Kikuyama, 1986*b*). Taking all these results together, it can be concluded that the function of prolactin cells is enhanced at the advanced climax stage. The physiological significance of the increased levels of prolactin at this stage is uncertain, but it is probable that prolactin is involved in structural and functional development for postmetamorphic life. We have demonstrated that stimulation rather than release from inhibition by the hypothalamus is necessary for the prolactin surge at late climax (Kawamura, Yamamoto, & Kikuyama, 1986). One of the major stimulatory factors involved in the hypothalamus is considered to be thyrotropin-releasing hormone (Seki, Kikuyama & Suzuki, 1988). The cDNA probe obtained in the present experiment is expected to be useful for analysing the control mechanisms of prolactin synthesis in amphibians.

ACKNOWLEDGEMENTS

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