

Molecular cloning of growth hormone encoding cDNA of Indian major carps by a modified rapid amplification of cDNA ends strategy

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A modified rapid amplification of cDNA ends (RACE) strategy has been developed for cloning highly conserved cDNA sequences. Using this modified method, the growth hormone (GH) encoding cDNA sequences of *Labeo rohita*, *Cirrhina mrigala* and *Catla catla* have been cloned, characterized and overexpressed in *Escherichia coli*. These sequences show 96–98% homology to each other and are about 85% homologous to that of common carp. Besides, an attempt has been made for the first time to describe a 3-D model of the fish GH protein.

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1. Introduction

1.1 Growth hormone

Growth promoting function of the pituitary gland was first described in 1921 (Evans and Long 1921) and the human growth hormone (GH) encoding cDNA was perhaps the first to be isolated and characterized (Li and Evans 1944). GH, chorionic somatomotropin (placental lactogen) and prolactin (PRL) are all a family of hormones, thought to have evolved from a common precursor (Nial *et al* 1971; Miller and Eberhardt 1983). Growth hormone controls the postnatal somatic growth (Reiter and Rosenfeld 1998; Sakamoto *et al* 1993) and reproduction (Van der Kraak *et al* 1990; Le Gac *et al* 1993) in vertebrates. These functions of GH are mediated by insulin like growth factors (IGF 1 and 2, Mathews *et al* 1986; Sara 1991) and growth related protein kinases (Ralph *et al* 1990).

The importance of the GH as a potential growth promoting agent has long been recognized and GH adminis-

tration has been shown to accelerate growth rate in a number of animals, especially fish (Cavari *et al* 1993; McLean *et al* 1993; Tsai *et al* 1994; Agelon 1998). Hence the cloning, characterization and expression of GH have been the subject of extensive research (e.g. Chang *et al* 1992; Venugopal *et al* 1998) during the last decade. Due to its importance in fish culture, GH encoding cDNA has so far been cloned from about 30 fish species (Bernardi *et al* 1993; Pandian and Marian 1994; Pandian *et al* 1999). However, almost all the described GH sequences are for fishes of Europe and western countries. The Indian carps namely rohu (*Labeo rohita*), mrigal (*Cirrhina mrigala*) and catla (*Catla catla*), collectively called the Indian major carps, constitute the most important cultivable freshwater fishes of India. In this study, the GH encoding cDNA of mrigal and catla have been amplified by following a modified rapid amplification of cDNA ends (RACE) strategy, namely homology based-RACE (H-RACE), subsequently cloned, characterized and expressed in *Escherichia coli* using a his-tag vector. Our earlier findings on rohu GH encoding are taken for

Keywords. cDNA cloning; growth hormone; Indian major carps; overexpression; protein 3-D structure; RACE

Abbreviations used: GH, Growth hormone; GTC, guanidium thiocyanate-phenol-chloroform; H-RACE, homology based-rapid amplification of cDNA ends; PRL, prolactin; RACE, rapid amplification of cDNA ends.

comparative purpose (see Venugopal *et al* 2002). Besides, an attempt has also been made for the first time to describe the 3-D structure of rohu GH.

1.2 RACE strategy

Despite the development of numerous cDNA cloning strategies (Gubler and Hoffman 1983; Okayama and Berg 1982), obtaining the full-length cDNA copies of scarcely abundant mRNA has been a formidable task (Frohman *et al* 1988). Most methods involved in cDNA library construction require either 5 µg or more of poly A⁺ RNA (e.g. ≥ 10 µg, Rivolta and Wilcox 1995), or a large amount of total RNA (Schraml *et al* 1993). This may prove a major task (Ghosh 1996) when an expensive or scarce cell line or tissues like fish pituitary is to be used. Moreover the conventional cDNA library construction procedures are cumbersome and involve screening of thousand of recombinant phages (Sambrook *et al* 1989), which is time consuming and laborious process. To circumvent this difficulty, novel and relatively simple polymerase chain reaction (PCR) based methods, namely RACE and one sided polymerase reaction were developed by Frohman *et al* (1988) and Ohara *et al* (1989), respectively. This strategy requires a meagre amount of total RNA, the information of which is copied to DNA by reverse transcriptase, and subsequently amplified by PCR with either gene specific (Frohman *et al* 1988) or degenerate (Ohara *et al* 1989) primers to generate required quantities of DNA for cloning into plasmid vectors. Lemaire *et al* (1994) are perhaps the only group to adopt the method of Ohara *et al* (1989) to successfully amplify the GH cDNA of the giant freshwater catfish *Pangasionodon gigas*, but as two separate DNA fragments. These two DNA fragments need to get spliced together, in an in-frame fashion to generate functional cDNA, which is a time tedious step. To overcome this difficulty a simple new strategy, H-RACE has been developed to amplify the full length GH cDNA of fish, as a single DNA fragment.

2. Materials and methods

2.1 Dissection and isolation of pituitary glands

The skull of a 3–6 month old, freshly collected carp was cut-opened from the dorsal side. Without contamination of other tissues, the pituitary gland was scooped out and preserved immediately in guanidium thiocyanate-phenol-chloroform (GTC) solution (Chomczynski and Sacchi 1987). For preservation of every 50 glands, 1 ml of GTC was used. The pituitaries were homogenized and brought to laboratory on ice, where the RNA extraction process was resumed immediately.

2.2 RNA extraction

Total cellular RNA was extracted following standard procedure (Chomczynski and Sacchi 1987). All solutions were prepared from DEPC-treated autoclaved, distilled water. Care was taken to avoid contamination at any stage such as pituitary collection, homogenization and RNA extraction. The RNA pellet was dissolved in DEPC-treated autoclaved, distilled water and stored at – 70°C.

2.3 H-RACE strategy

By multiple sequence alignment, available fish GH protein sequences were compared, and a conserved domain was identified at the C terminal end. Based on this conserved domain, degenerate PCR primers namely G1 and G2 were designed following the strategy of Lemaire *et al* (1994). Using the primer G1 and poly T primer, 3' end of the GH encoding cDNA of the 3 Indian carps were amplified by RT-PCR. These GH cDNA were subsequently cloned and characterized. The partial sequences were compared with the available fish GH cDNA sequences by multiple sequence alignment, and the highly homologous sequences were selected. The selected sequences were analysed and a conserved domain was identified at the most upstream regions. Based on this conserved region, another primer namely G4 was designed. Using G4, which binds at the 5' most region and the polyT primer, which binds to the 3' most region, the full length GH cDNA of the Indian carps was amplified as a single DNA fragment.

The fidelity of the H-RACE method was verified by taking rohu GH cDNA sequence as example. The rohu GH cDNA generated by H-RACE in the present study and the GH cDNA sequence generated from pituitary cDNA library clones of rohu (Venugopal *et al* 2002) were compared.

2.3a Cloning of the 3' end partial GH cDNA: The first strand cDNA was synthesized by a modified protocol of Ausubel *et al* (1999). Total RNA (~ 2 µg in 10 µl) was incubated at 65°C for 10 min with poly T primer (CGG AAT TCT AGA (T)₁₈, – 30 mer). The 3' end GH cDNA was amplified with degenerate G1 primer (5' CGG AAT TCA TCG ACA ARG TSG AGA C 3' – 25 mer; R = A or G; S = C or G) following a similar method adopted for the 3' cDNA cloning of rohu GH (Venugopal *et al* 2002). The G1 primer is expected to bind with the GH mRNA at the 3' end as to amplify a ~ 600 bp segment. The synthesized first strand cDNA (equivalent of 200–300 ng total RNA) was subsequently amplified by PCR. The 3' end of the GH cDNA was amplified using Taq DNA polymerase with 10 pmol of G₁ and poly T primers (per 100 µl) in a thermal cycler (PerkinElmer 2400). The PCR

product corresponding to the 3' end of the GH mRNA was purified by passing it through a DNA purification column (Qiagen, Germany) and ligated to a T-tailed pMosBlue vector (Amersham, USA).

2.3b Cloning of full length GH cDNA: A comparison of the 3' end of the rohu GH cDNA sequence with other available DNA sequences by Pearson and Lipman algorithm using the GCG software showed the maximum homology with cyprinid GH cDNA sequences. Such GH cDNA sequences, which show maximum homology with that of rohu, were selected and grouped. This group of sequences was compared by multiple sequence alignment, and a conserved block was identified at the 5' most region of the GH cDNA (figure 1). Based on this observation, a degenerate primer, namely G4 (GAA TTC CTA CCC TGA NCG AAA TGG CTA GAG – 30 mer), which binds at the 5' most end of the first strand (-ve strand) GH cDNA was designed. The column purified first strand cDNA were amplified with 10 pmol each of poly T and G4 primers by PCR. The PCR protocol was as follows: denaturation at 94°C for 40 s, annealing at 60°C for 90 s, primer extension at 72°C for 3 min. Thirty cycles of reactions were performed with an initial denaturation at 94°C for 2 min and final extension of 15 min at 72°C. The resulting PCR products were analysed on a 2% agarose

gel, blunted and cloned in a pMosBlue blunt vector (Amersham) following standard protocols.

2.4 DNA sequencing

Column purified/Miniprep extracted plasmid DNA were sequenced in automated DNA sequencer using Big Dye terminator kit (Perkin Elmer), from both the ends using universal (T3 and U19) primer and internal regions were sequenced by sequence walking method with custom synthesized GH specific primers.

2.5 Sequence analysis

Nucleotide and translated amino acid sequences were analysed by FastA module of the GCG package (Genetics Computers Group, version 9-2, Wisconsin University, USA) and BLAST searches (<http://www.ncbi.nlm.nih.gov/blast>). The percentage identity between sequence pairs was calculated using GAP (GCG). Multiple sequences were aligned using PILEUP (GCG) and CLUSTAL W (1-60). ORFs were identified using ORF FINDER of EMBL (<http://www.ebi.ac.uk>) and FRAMES (GCG). Protein sequences were analysed by different modules of the GCG software. GeneDoc software was used for analysing similarity and scoring among the aligned sequences.

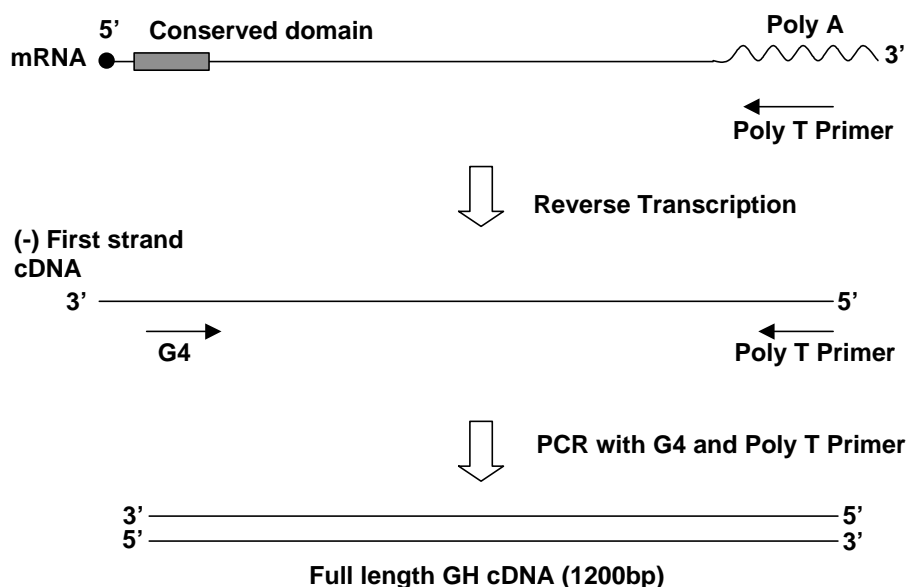


Figure 1. Modified RACE strategy adopted to amplify the full length GH cDNA of Indian carps. Partial GH cDNA (3' end) was amplified by following a similar strategy of Lemaire *et al* (1994). The partial sequence was compared with available sequences by multiple alignment. The sequences, which were considerably homologous to the partial GH cDNA sequences of carps were selected and grouped. This group of sequences was analysed, and a conserved region was identified at the most upstream region based on which a primer namely G4 was designed. Full length GH cDNA of carps were amplified using poly T and G4 primers from the first strand cDNA template.

2.6 Secondary and tertiary structure prediction

Secondary structures of the proteins were determined by inhomogenous score combination method of Guermeur *et al* (1999) based of neural networks (<http://npsa-pbil.ibcp.fr/npsa>) and by the PROFILESCAN, PEPTIDESORT and other modules of the GCG software. Tertiary structure of the proteins were predicted by homology based modelling software of the SWISSPROT (<http://swissprot.ch>) and were analysed by RASWIN (version 2.7.1).

2.7 Overexpression of GH protein in *E. coli*

2.7a Construction of His-tag vectors for expression of GH in *E. coli*: GH encoding ORF of the 3 carps was amplified from their respective full length clones by PCR, using similar primers [forward primer containing *Bam*HI site (CGC GGA TCC TCA GAT AAC CAG CGG – 24 mer) and reverse (CCC AAG CTT CAG GGT GCA GTT GGA – 24 mer) primer containing *Hind*III site], used for the GH expression of rohu (Venugopal *et al* 2002). Each of the PCR products was separately digested with respective enzymes and cloned into pQE30 His-tag vector (Qiagen, Germany). The vector contains 6 His residues at the N terminus that facilitates the GH protein purification (Hochuli 1988) by immobilized metal affinity chromatography (IMAC). The ligation reactions were transformed into XL1 blue cells and recombinants were characterized. The plasmids containing the GH encoding ORF of these carps were transformed into M15 cells (containing pREP4 plasmid) and recombinants were characterized.

2.7b Overexpression of GH: *E. coli* transformant cells (M15) harbouring the pQmGH and pQcGH plasmid were inoculated into a 5 ml LB medium containing ampicillin and kanamycin, and were grown overnight at 37°C with shaking. LB medium (50 ml) was inoculated with 0.1% of overnight inoculum and grown at 37°C. When cells reached 0.6 at A_{600} , the culture was induced with 0.75 mM IPTG and allowed to grow for 2 more hours. The cells were harvested and lysed in a buffer containing 8 M urea, following the standard protocols of the manufacturer. The protein was estimated (Lowry *et al* 1951) and analysed by 12.5% SDS-PAGE following standard protocols (Laemmli 1970).

3. Results

3.1 Cloning of partial GH cDNA of Indian carps

The PCR amplification of the first strand cDNA of the carps with G1 and poly T primers yielded a product of almost a similar size, 590 bp each. The PCR amplified 3'

end GH cDNA of mrigal and catla were cloned into a T-tailed plasmid vector to generate pMos Δ mGH and pMos Δ cGH, respectively, which were sequenced. The sequence showed a high degree of homology with the GH encoding sequences of *Cyprinus carpio* Linn. and other carps, confirming that the 590 bp insert is indeed the partial GH cDNA.

3.2 Cloning of the full length GH cDNA of Indian carps

PCR amplification of the first strand cDNA of all the 3 carps yielded a single discrete PCR product of ~ 1200 bp each, the expected size. The PCR products of rohu, mrigal and catla were separately purified and subsequently cloned into a T-tailed plasmid vector (figure 2) to generate pMos rGH, pMos mGH and pMos cGH respectively, and sequenced. The peptide sequence of the cDNA sequences showed somatotropin, prolactin and

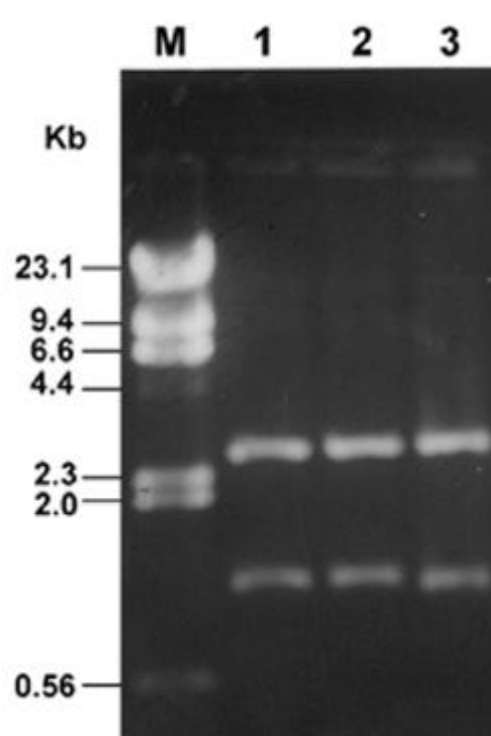


Figure 2. Cloning of the full length GH cDNA of the Indian major carps, generated by novel H-RACE strategy. The H-RACE generated full length GH cDNA of rohu, mrigal and catla were blunted with Pfu DNA polymerase and ligated to blunt end vector to generate pMosrGH, pMosmGH and pMoscGH, respectively. These plasmids were digested with *Eco*RI and resolved on 0.7% agarose gel containing ethidium bromide. Lane M, *Hind*III marker; lane 1, pMosrGH digested with *Eco*RI; lane 2, pMosmGH digested with *Eco*RI; lane 3, pMoscGH digested with *Eco*RI.

related signatures (Wallis 1981), which confirmed that the amplified DNA fragments indeed encode for the growth hormone.

3.3 Growth hormone cDNA of mrigal

The GH encoding cDNA of mrigal consists of 1146 nucleotides (figure 3). It has a 14 bp UTR at the 5' region and a 502 bp UTR at the 3' end. The ORF spans from the 15th base to the 648th base, which codes for a protein of 210 amino acids, including a signal peptide of 22 amino acids. The nucleotide sequence of mrigal is 97% identical to that of rohu and 96% identical to that of catla.

Molecular weight of this GH protein is 23.7 kDa, and the isoelectric point is 6.74. It has two putative glycosylation sites at 135 and 187 positions. The Cystein residues, which are important for the disulfide bond formation and structural integrity of the 3-D structure of the GH

protein (Watahiki *et al* 1989) is conserved. Cystein residues are located at 49, 161, 178 and 186 positions. In addition it has an extra Cys residue at position 123, like the GH proteins of carps (Chang *et al* 1992). Overall, the mrigal GH protein is longer than that of rohu by 3 residues and shorter than that of catla by one residue (table 1).

3.4 Growth hormone cDNA of catla

Catla GH cDNA is of 1156 bp in length (figure 4) and is the longest of all the 3 Indian carps. It has a 14 bp UTR at the 5' end and a 491 bp UTR at the 3' end. The ORF starts at the 15th base and spans up to the 650th base. The ORF codes for a peptide of 211 amino acids including a signal peptide of 22 amino acids. The nucleotide sequence of catla is 98% identical to that of rohu and 96% identical to that of mrigal. A comparison of amino acid sequences of cyprinid growth hormones is given in the table 2.

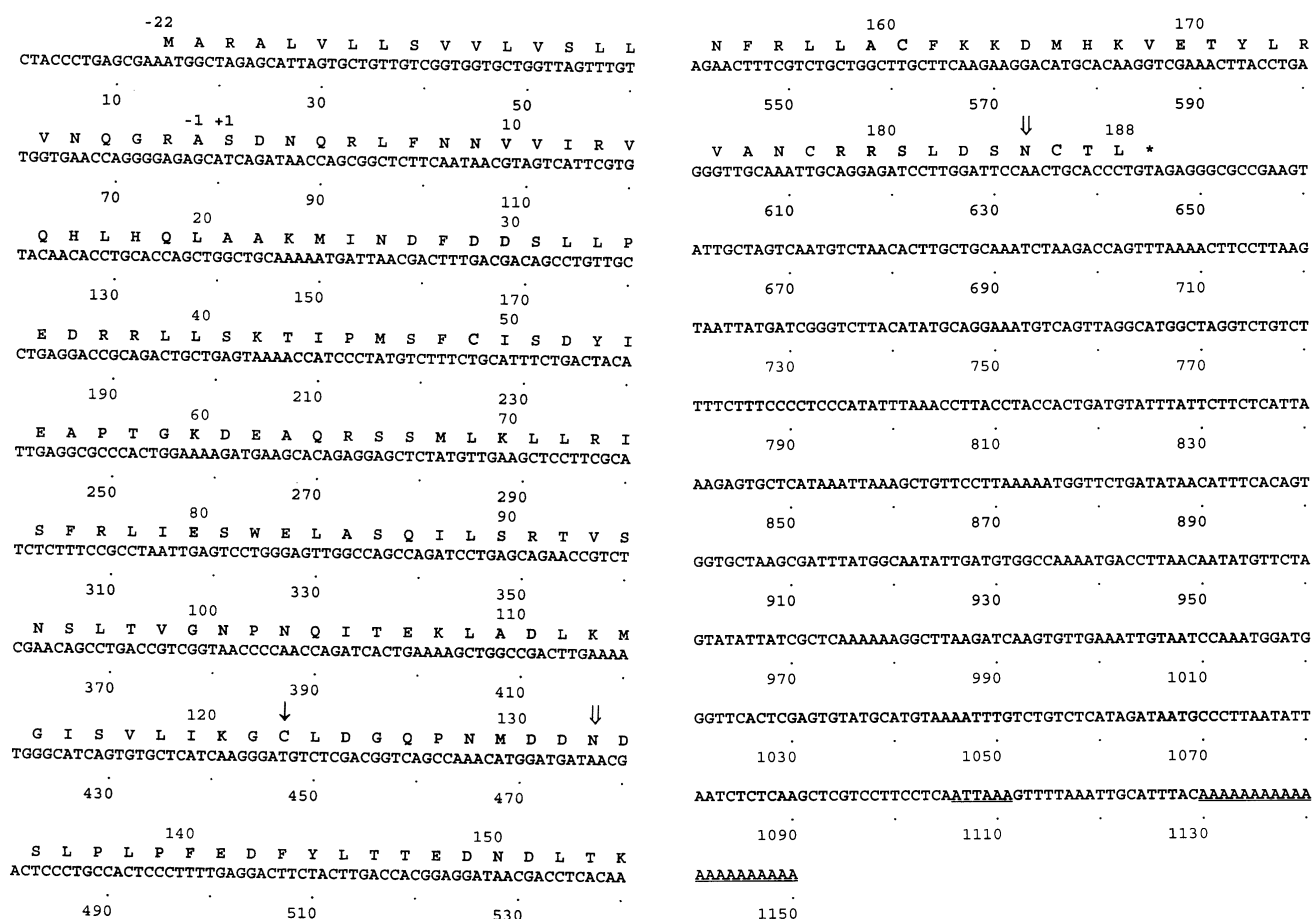


Figure 3. The complete cDNA sequence encoding for mrigal preGH protein. The mrigal GH comprises a signal peptide (-22 to -1) and the mature GH peptide (1 to 188). It has 2 N-glycosylation sites (Asn-Glu-Ser and Asn-Cys-Thr) which are marked by ↓ symbol. The poly adenylation signal and poly A tail are underlined. Cys residues are shown in enhanced fonts. An inverted arrow (↓) shows the unusual Cys of the cyprinid signature (Genbank accession No: AF140281).

The catla GH protein is 23.9 kDa in molecular weight with an isoelectric point of 6.5, and has two putative glycosylation sites at positions 135 and 188. The protein is conserved with 4 Cystein residues at 49, 161, 178 and

187 positions, like any other GH protein (Watahiki *et al* 1989). Catla GH protein is longer than that of mrigal by one residue and longer than that of rohu by four residues. The GH protein of these carps contains an

Table 1. Comparison of the GHs of the Indian major carps. The three Indian carps share > 96% homology with each other.

Character	Rohu	Mrigal	Catla
Nucleotides	1146	1150	1156
5' UTR	14	14	14
ORF	624	633	636
Stop codon	TAG	TAG	TAG
AT ratio	57:21	57:21	56:74
Protein	207aa	210aa	211aa
Signal peptide	22aa	22aa	22aa
Mol. Wt (kDa)	2320.86	2379.19	2390.22
Isoelectric point (pH)	6.74	6.74	6.50
Glycosylation sites	130, 182	133, 185	133, 186
Cystein residues	49, 120 , 158, 175, 183	49, 123 , 161, 175, 186	49, 123 , 161, 178, 187

The cypyrinid specific Cys is indicated in bold letters.

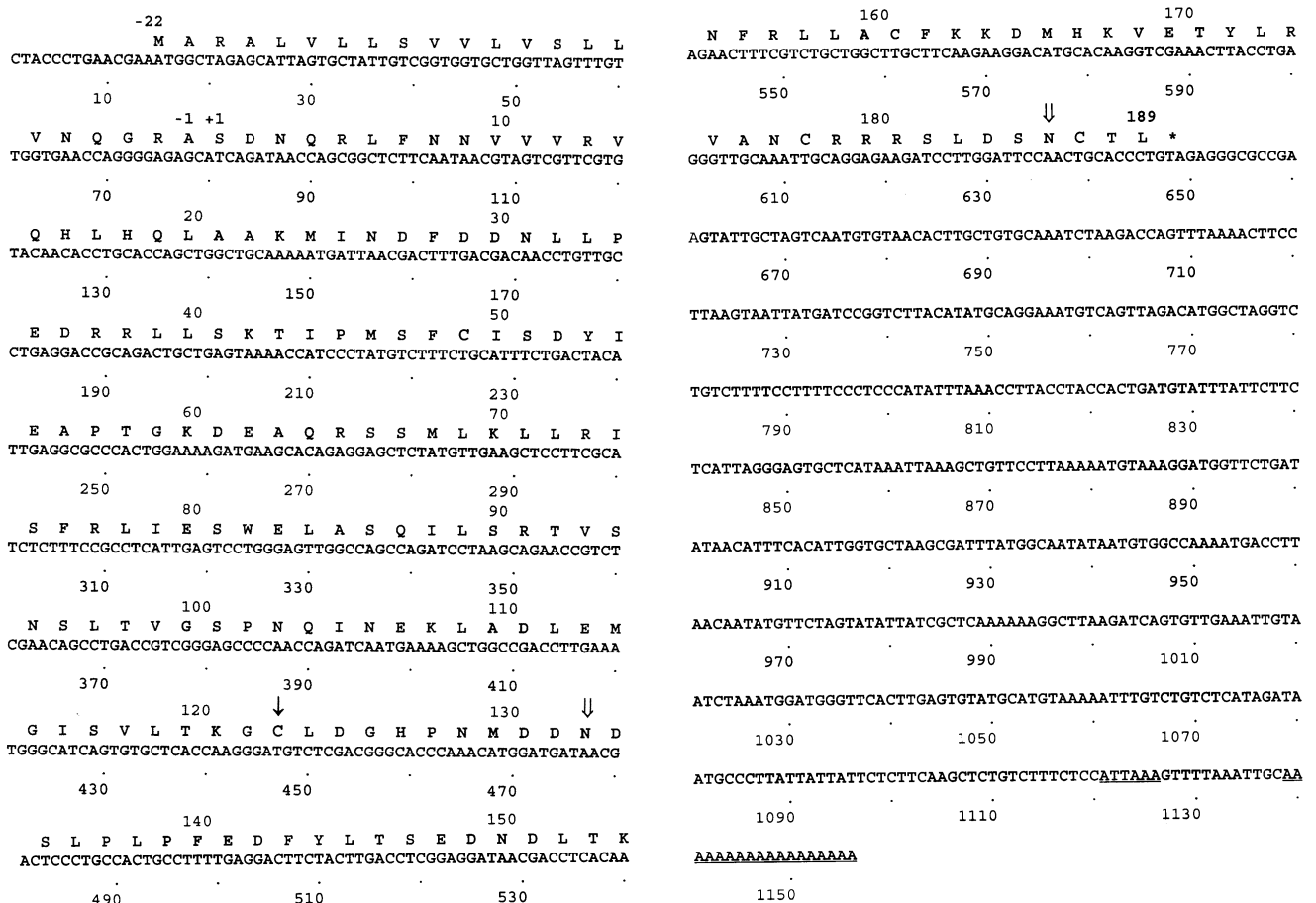


Figure 4. The complete cDNA sequence encoding for catla preGH protein. The catla GH comprises a signal peptide (– 22 to – 1) and the mature GH peptide (1 to 189). It has 2 N-glycosylation sites (Asn-Glu-Ser and Asn-Cys-Thr) which are marked by ↓ symbol. The poly adenylation signal and poly A tail are underlined. Cys residues are shown in enhanced fonts. An inverted arrow (⇓) shows the unusual Cys of the cypyrinid signature (Genbank accession No: AF140282).

extra Cys residue, like other cyprinid fishes (Chang *et al* 1992).

3.5 3-D structure of the growth hormones

The 3-D structure of the rohu GH protein (figure 5) is similar to that of known GH proteins of mrigal and catla,

Table 2. Homology of GH protein of *C. mrigala* with other cyprinids.

Species	Genbank accession	Similarity (%)
<i>Labeo rohita</i>	AF134200	98.6
<i>Catla catla</i>	AF140282	96.7
<i>Cyprinus carpio</i>	X13670	92.4
<i>Hypophthalmichthys nobilis</i>	X60473	92.4
<i>Ctenopharyngodon idella</i>	M27000	88.9

and the human GH protein, and has all the salient features of a typical 4-helix bundle protein described by Kohn *et al* (1997) and Carlacci *et al* (1991). Briefly, more than 55% of the rohu GH polypeptide chain are in the form of α -helix, folded to give four helices (Carlacci and Chou 1990) in an anti-parallel twisted helical bundle. The GH peptides of mrigal and catla have an additional stretch of three residues, which are in contrast to that of rohu and fall on the random coil region. A comparison of the rohu GH sequence with that of other fish GH reveals that the α -helical regions (figure 6) are more conserved.

3.6 Overexpression of the GH protein

The GH encoding ORF of mrigal and catla were separately amplified and cloned into His-tag vectors, which were named pQEmGH and pQEcGH, respectively (figure 7). Analysis of the protein extracted from the

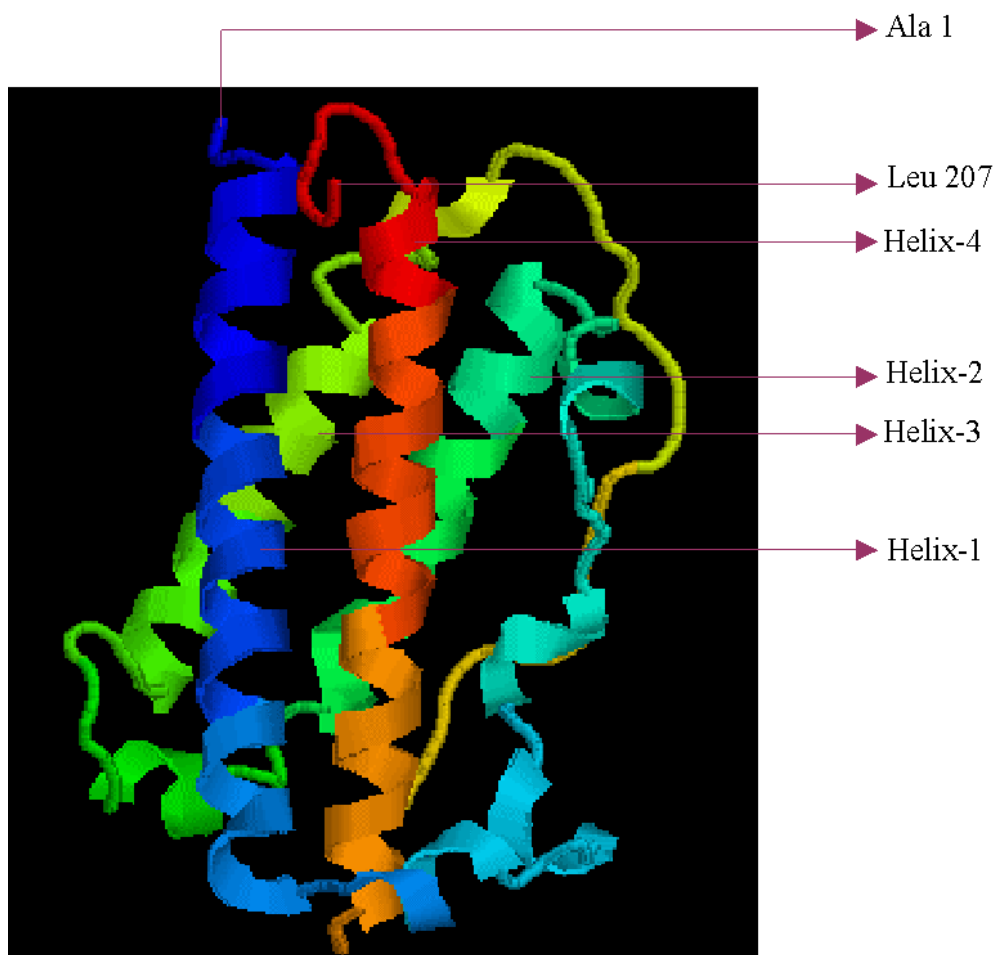


Figure 5. 3-D structure of the rohu GH. Out of 207, 118 amino acids (57%) are involved in α -helix formation, which run anti-parallel to each other. These helices attribute to the typical 4- α -helix bundle protein conformation, the characteristic 3-D confirmation of growth hormones.

protein is totally conserved in a few genera of fishes, which exhibit 100% identity. The three different genera namely *Oreochromis*, *Oncorhynchus* and *Hypophthalmichthys*, which belong to different orders, are closely related in terms of their GH sequences. In as much as the GH genes are highly conserved, the GH protein of these three genera are also identical, i.e. 100% homologous (Bernardi *et al* 1993). Despite this close genetic relation, there is no report till date about the inter-generic hybridization between these three genera, which may not be possible for following reasons. *Oncorhynchus* is geographically isolated from the others, and the obligate need for oral brooding by the parents excluded the scope of hybridization of *Oreochromis* with *Hypophthalmichthys*. It is interesting to note that the three different genera of the Indian carps hybridize freely with each other, despite their genetic relation being only 96–98%, in terms of GH protein sequences.

Due to its importance in biology, and application in medicine and animal husbandry, there is considerable significance in exploring the molecular features of GH

(Abdel-Meguid *et al* 1987; Cunningham *et al* 1991; De Vos *et al* 1992; Somers *et al* 1994). Based on the circular dichroism and intrinsic fluorescence spectroscopic observations, Hara *et al* (1978) proposed the first three-dimensional structure of bovine GH in a two-dimensional representation, by applying the Chou-Fasman protein structure prediction method. After a decade the crystal structure of the porcine GH molecule was solved by Abdel-Meguid *et al* in 1987. Our present knowledge on the 3-D structure of GH proteins is limited to pig (Abdel-Meguid *et al* 1987), cow (Carlacci *et al* 1991) and human (Cunningham *et al* 1991; De Vos *et al* 1992; Somers *et al* 1994).

In all the known crystallographic and theoretical GH structures, more than 50% of the residues are involved in the formation α -helices in anti-parallel twisted helical bundle. Sequence comparison indicates that the α -helical regions are more conserved than other parts of the molecule (Wallis 1989), suggesting the possibility of similar conformation in all the other proteins of the GH family. Members of the GH family possess either two (GH) or

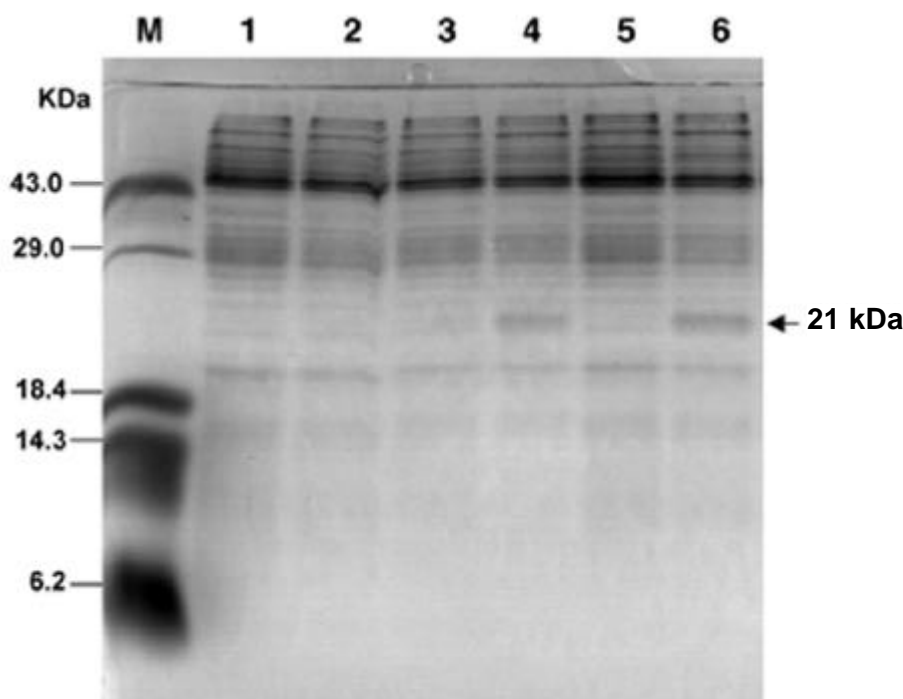


Figure 8. Expression of GH proteins of mrigal and catla in *E. coli*. The cells harbouring the pQmGH and pQcGH were grown and induced with 0.75 mM of IPTG. The cells were harvested 2 h after induction and total protein was extracted with urea buffer. Proteins were resolved by SDS-PAGE (12.5%) and stained with Comassie blue, and destained. The induction of a 23 kDa protein in IPTG induced pQmGH/pQcGH samples (lanes 4 and 6) is indicated by an arrow. Lane M, Protein molecular weight marker; lane 1, cells harbouring pQ30 vector before induction; lane 2, Induced cells harbouring PQ30 vector; lane 3, cells harbouring pQmGH vector before induction; lane 4, cells harbouring pQmGH vector after induction; lane 5, cells harbouring pQcGH vector before induction; lane 6, cells harbouring pQcGH vector after induction.

three (PRL) disulfide bonds, which are highly conserved. For instance, the Cys residues involved in disulfide bonds are located in positions 53, 164, 181 and 189 in bovine GH. When this sequence is aligned to optimize amino acid similarity with other GH sequences, the four Cys residues are conserved in all the GH and PRLs.

The GH protein of all the vertebrates species contain four Cys residues that render the functional 3-D confirmation of the hormone by establishing two intramolecular disulfide (S-S) bonds. As an interesting exception, the GH of all fish species studied to date which belong to the family 'Cyprinidae' contain five Cys residues. Recent studies suggest that the first disulfide bond is formed between Cys49 and Cys161, and the second between Cys178 and Cys186 (Vestling *et al* 1991). In addition to the four residues, which occur at positions similar to those of other vertebrates (Chang *et al* 1992) the fifth residue is located at the position 120 of rohu GH. Thus their equivalents Cys 123 of mrigal and catla, and Cys 120 of rohu may not be required for the proper folding of the cyprinid GH proteins. This assumption is supported by investigations of Fine *et al* (1993), wherein the Cys123 of carp GH by Ala was replaced through site directed mutagenesis and the functional properties of the protein was studied by expressing the mutant protein in *E. coli*. The results showed that the mutated GH protein could effectively attain its 3-D confirmation by forming legible disulfide bonds, but exhibited lower binding affinity to the GH receptors in liver than the normal GH protein. The mutated GH protein showed 12 times lower biological activity in term of inhibition of DNA synthesis, which was determined by measuring the [³H]thymidine incorporation in 3T3-F442 rat preadiposites (Corin *et al* 1990). The unique case (Mahmoud *et al* 1996) of all the cyprinid GH proteins studied to date is the goldfish GH, which has Ser instead of Cys at position 123. However this exception does not exclude the possibility of the presence of a second GH with five Cys residues in the goldfish (Mahmoud *et al* 1996) as a different isomer (Aramburo *et al* 1991).

In contrast to mammalian GH proteins, the fish GH proteins contain a polar amino acid at position 123, which is replaced by a nonpolar amino acid. For instance, the GH of salmon and seabream contain a Ser and Asn at position 123, but the human GH contains a nonpolar (Leu) residue at this position (Mahmoud *et al* 1996). Hence like all other piscine GH proteins the cyprinid GH proteins too require a polar amino acid at position 123 and not necessarily a Cys. Occurrence of a Ser at position 123 in goldfish supports this proposition (Mahmoud *et al* 1996).

The amino acid sequence homology among the GH proteins of fishes suggests that the GH proteins of other fishes might share a similar 3-D structure. Since the

current 3-D model is the first one for a fish GH protein and only homology-based, further discussion on the model is not possible.

Glycosylation is an important aspect of the GH protein, as it has 2 potential glycosylation sites, which generate different forms of the GH protein. For instance, the GH secreted in the pituitary gland (hGH) of human is not glycosylated but that of the placenta during pregnancy is glycosylated. In fish, glycosylation of the GH protein is reported in salmon (Wagner *et al* 1985), but whether there is a corresponding glycosylation in carps is not yet known (Chang *et al* 1992).

5. Conclusions

The GH encoding cDNA of the three Indian major carps have been amplified by following a new but modified H-RACE, cloned, sequenced and characterized.

The fidelity of the H-RACE generated GH encoding cDNA of these carps was counter-checked by taking the rohu GH cDNA as example. The H-RACE generated clone of rohu was compared with the GH cDNA sequence of cDNA library generated clone. A cDNA library was constructed from the poly A⁺ RNA extracted from the pituitary glands of rohu (Venugopal *et al* 2002). The library was screened with the partial 3' end GH cDNA as probe. The GH cDNA was retrieved from the hybridization positive plaques by *in vivo* excision as a plasmid vector, and sequenced with universal and GH specific primers. The nucleotide sequences were compared by Gap program of the GCG software.

The H-RACE generated and the library generated sequences showed 100% identity, except that the library generated clones contain a 45 bp 5' UTR in contrast to the 34 bp 5' UTR of H-RACE generated clones. The results show that the strategy of new H-RACE could be useful to amplify highly conserved cDNA sequences.

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