

**EXPRESSION STUDIES OF TWO HYPOXIA-INDUCIBLE FACTOR  
(HIF- $\alpha$ ) GENES FROM THE GRASS CARP,  
*CTENOPHARYNGODON IDELLUS***

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

In mammals, hypoxia-inducible factor 1 (HIF-1) is a key transcription factor that controls a variety of cellular and systemic homeostatic responses to hypoxic stress by upregulating the expression of genes involved in enhancing O<sub>2</sub> delivery, glucose uptake and glycolysis (Semenza, 2001). HIF-1 is a dimeric basic helix-loop-helix (bHLH) protein composed of HIF-1 $\alpha$  and HIF-1 $\beta$  subunits. In vertebrates, the HIF-1 $\alpha$  protein and its DNA-binding activity is tightly regulated by O<sub>2</sub> concentration and is increased as O<sub>2</sub> level decreases (Jiang et al., 1996). So far, two different HIF- $\alpha$  cDNAs have been reported in fish – HIF-1 $\alpha$  in *Oncorhynchus mykiss* (Soitamo et al., 2001) and HIF-2 $\alpha$  in *Fundulus heteroclitus* (Powell and Hahn, 2002).

In order to investigate the functional roles of HIF- $\alpha$  in the hypoxia-tolerant grass carp, we have isolated two different full-length HIF- $\alpha$  cDNAs of 3.9 and 2.1 kb from primary kidney cells of grass carp using degenerate RT-PCR and, 5'- and 3'-RACE. Computer analysis indicated that the ORF of the 3.9-kb cDNA encodes for a putative protein of 774 amino acids which shares > 70% sequence similarity with the mammalian HIF-1 $\alpha$  proteins. This cDNA was therefore believed to encode for the grass carp HIF-1 $\alpha$  (gcHIF-1 $\alpha$ ). On the other hand, analysis of the 2.1-kb cDNA showed that it encodes for a putative protein of 643

amino acids, which is equally similar in sequence (~ 50%) to the HIF-1 $\alpha$ , -2 $\alpha$  and -3 $\alpha$  proteins from different vertebrate species. This cDNA was believed to encode a novel HIF- $\alpha$  isoform and was therefore named gcHIF-4 $\alpha$ . Both deduced proteins contain the characteristic bHLH, Per-Arnt-Sim (PAS) and oxygen-dependent degradation (ODD) domains typically found in HIF- $\alpha$  proteins (Figure 1).

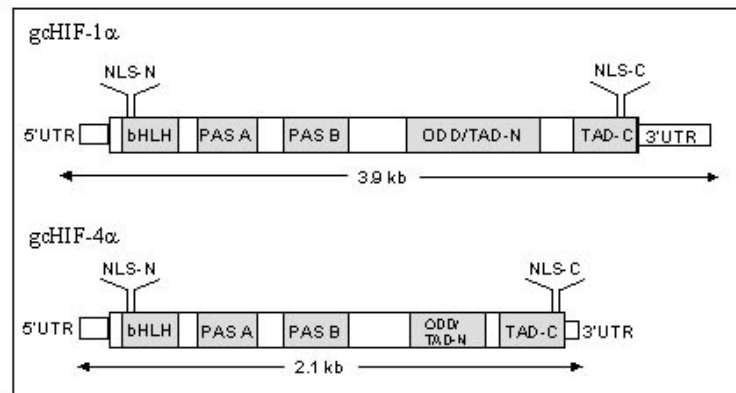


Figure 1. Deduced protein structures of gcHIF-1 $\alpha$  and gcHIF-4 $\alpha$  cDNAs. UTR, untranslated region; bHLH, basic helix-loop-helix domain; PAS A/B, Per-Arnt-Sim domain; ODD, oxygen dependent degradation domain; TAD-N/-C, transactivation domain – N-terminal/C-terminal; NLS-N/-C, nuclear localization signal – N-terminal/C-terminal.

To study the tissue distribution and expression patterns of *gcHIF-1 $\alpha$*  and *gcHIF-4 $\alpha$* , grass carps (n = 3) were exposed to hypoxic (0.5 mg/L DO) and normoxic (7 mg/L DO) conditions for 4 hours, and total RNA was isolated from 8 different tissues of each fish for Northern blot analysis. In all tissues examined, with the exception of the heart, *gcHIF-4 $\alpha$*  is expressed at higher levels than *gcHIF-1 $\alpha$* , with the highest expression detected in muscle (Figure 2). Upon exposure to hypoxia for 4 hrs, transcription of *gcHIF-1 $\alpha$*  was significantly downregulated in

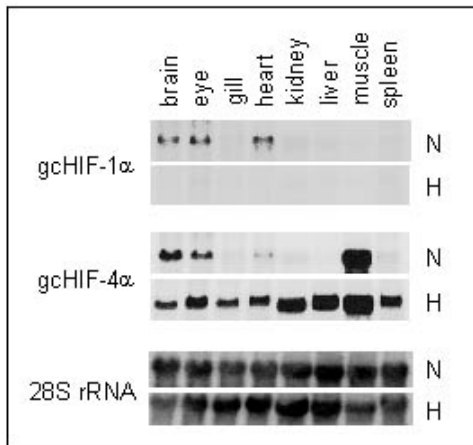


Figure 2. Northern blot analysis of *gcHIF-1α* and *gcHIF-4α* expression in eight different tissues of grass carps exposed to normoxia and hypoxia for 4 hours. Expression was normalized against the 28S rRNA. N = normoxia, H = hypoxia.

the brain, eye and heart. Expression of *gcHIF-1α* was only detectable in the gill, kidney, liver, muscle and spleen tissues when the autoradiogram was exposed for another 24 hrs (data not shown). In contrast, expression of *gcHIF-4α* was markedly upregulated in the eye, gill, heart, kidney, liver, spleen and muscle of grass carp under hypoxia. To verify this observation, Northern blot analysis was performed on total RNA samples from two other grass carps that were subjected to identical normoxic and hypoxic treatments, and results similar to those in Figure 2 were obtained.

In conclusion, expression of the *gcHIF-1α* gene is downregulated under hypoxia in various fish tissues indicating that its mode of regulation is markedly different from its mammalian homologues. This suggests that the *gcHIF-1α* protein is not involved in controlling the same gene targets as those in mammals. In contrast, *gcHIF-4α* is upregulated under hypoxia but whether or not the *gcHIF-4α* protein is involved in upregulating the genes involved in enhancing O<sub>2</sub> delivery, glucose uptake and glycolysis awaits further functional studies.

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