

**ISOLATION AND CHARACTERIZATION OF A HYPOXIA-
RESPONSIVE GLUCOSE TRANSPORTER GENE FROM GRASS CARP**

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Glucose transporters (GLUTs) are a family of glycosylated membrane proteins that facilitate passive uptake of hexose into the cell. In humans, a family of 13 GLUT proteins with distinct tissue distribution and physiological functions have been described. On the basis of sequence similarities and characteristic signature motifs, they are divided into three subfamilies – class I consists of glucose transporters GLUT1-4; class II consists of GLUT5, 7, 9 and 11; and class III consists of GLUT6, 8, 10, 12 and the myo-inositol transporter (HMIT) (Joost and Thoren, 2001).

In mammals, an important physiological response in the face of reducing oxygen tension is to switch to anaerobic respiration. This entails the enhancement of glucose transport which is facilitated by upregulating *Glut1* expression in a variety of tissue types, and is mediated by the HIF-1 transcription factor (Ebert et al., 1995). Although four different GLUT cDNAs have so far been reported in fish – *Glut1* in common carp, *Glut1A* and *Glut2* in rainbow trout, and *Glut4* in brown trout – no information on the responses of these genes to hypoxia is available. Recently, we have isolated a genomic gene for the GLUT1 protein in grass carp. The *gcGlut1* gene is ca. 16 kb in length and contains 12 exons and 11 introns (Figure 1). Based on sequence information from one of the exons of *gcGlut1*, two full-length cDNAs of 2.1 and 3.0 kb were isolated by 5' and 3' RACE-PCR. Computer analysis indicated that the two cDNAs resulted from alternative use of polyadenylation sites in *gcGlut1*. This finding was corroborated by Northern blot analysis which also showed that the 3.0 kb mRNA is the major gene transcript expressed (data not shown). The ORF of

gcGlut1 encodes a putative polypeptide of 533 amino acids with a predicted molecular mass of ca. 57 kDa and a pI of 8.34. Homology search using Blastp showed that *gcGlut1* is most similar to the human (77% identity) and fish (75% identity) GLUT1 proteins. Alignment of the deduced amino acid sequence of *gcGlut1* with other GLUT proteins indicated a high degree of structural conservation and the presence of a number of characteristic sugar transporter signatures including: (1) the presence of 12 membrane-spanning helices; and (2) amino acid residues that are specific for class I sugar transporters (Joost and Thorens, 2001).

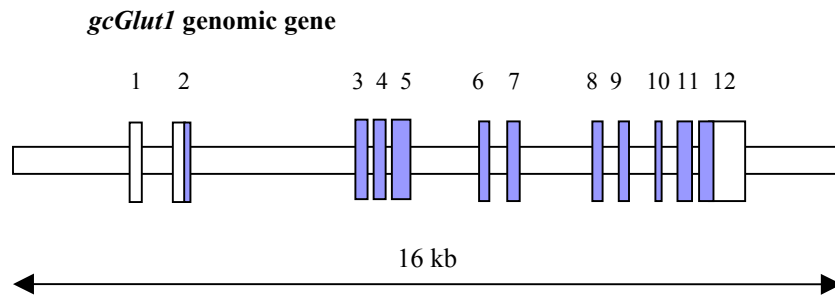
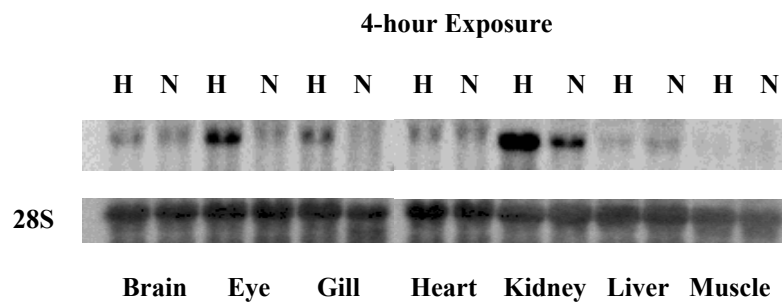


Figure 1. Genomic structure of *gcGlut1*. The twelve exons of the gene are shown as boxes and are numbered accordingly. Filled and open boxes indicate translated and untranslated regions, respectively.



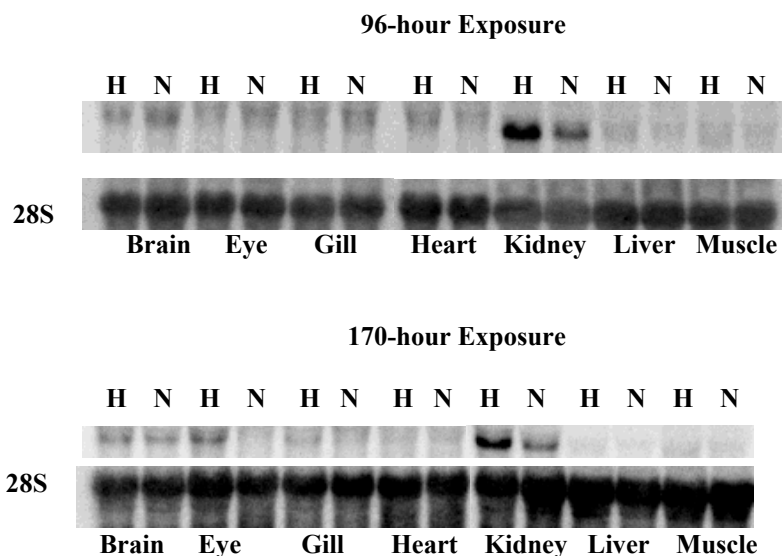


Figure 2. Responses of the *gcGlut1* gene to hypoxia in different organs of the grass carp. N = normoxia, H = hypoxia.

To study the tissue distribution and expression pattern of the *gcGlut1* gene, grass carps (n = 4) were exposed to hypoxic (0.5 mg/L DO) and normoxic (7 mg/L DO) conditions for 4, 96 and 170 hrs, and total RNA was isolated from seven different tissues for Northern blot analysis. A representative autoradiogram is shown in Figure 2. Under normoxic conditions, *gcGlut1* (3.0 kb transcript) is predominantly expressed in the kidney; however, low mRNA levels were also detected in brain, eye, gill, heart, liver and muscle. Hypoxia for 4 to 170 hrs resulted in a dramatic and persistent increase in *gcGlut1* transcript levels in the fish kidney, while hypoxic induction was only observed in the eye at 4 and 170 hrs, gill at 4 hrs and liver at 170 hrs. Expression of *gcGlut1* was seemingly unaffected in the heart and muscle of grass carp by hypoxia at all time points examined.

In conclusion, *gcGlut1* is predominantly expressed in the kidney of grass carp, which is also the organ where the highest increase in gene expression was observed during short term and prolonged hypoxia, suggesting that *gcGlut1* plays a fundamentally important homeostatic role in the kidney. Computer

analysis of the 5'-flanking sequence of the *gcGlut1* gene revealed the presence of several putative Hypoxia Responsive Elements (HREs) indicating that its expression is probably controlled by HIF-like transcription factors.

Reference

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