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RAINBOW TROUT GLUCOSE TRANSPORTER (OnmyGLUT1): FUNCTIONAL ASSESSMENT IN *XENOPUS LAEVIS* OOCYTES AND EXPRESSION IN FISH EMBRYOS

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Summary

Recently, we reported the cloning of a putative glucose transporter (OnmyGLUT1) from rainbow trout embryos. In this paper, we describe the functional characteristics of OnmyGLUT1 and its expression during embryonic development of rainbow trout. Transport of D-glucose was analysed in *Xenopus laevis* oocytes following microinjection of mRNA transcribed *in vitro*. These experiments confirmed that OnmyGLUT1 is a facilitative Na^+-independent transporter. Assessment of substrate selectivity, sensitivity to cytochalasin B and phloretin and kinetic parameters showed that the rainbow trout glucose transporter was similar to a carp transporter and to mammalian GLUT1. Embryonic expression of OnmyGLUT1 was studied using whole-mount *in situ* hybridization. Ubiquitous distribution of transcripts was observed until the early phase of somitogenesis. During the course of organogenesis, somitic expression decreased along the rostro-caudal axis, finally ceasing in the mature somites. The OnmyGLUT1 transcripts were detected in the neural crest during the whole study period. Transcripts were also found in structures that are likely to originate from the neural crest cells (gill arches, pectoral fins, upper jaw, olfactory organs and primordia of mouth lips). Hexose transport activity was detected at all developmental stages after blastulation. Cytochalasin B blocked the accumulation of phosphorylated 2-deoxy-D-glucose by dissociated embryonic cells, suggesting an important role for transport in glucose metabolism.

Key words: glucose transporter, GLUT1, *Xenopus laevis* oocyte, development, expression, rainbow trout, *Oncorhynchus mykiss*, *in situ* hybridization, kinetics.

Introduction

Passive glucose transporters (GLUTs) facilitate the passage of monosaccharides across the plasma membrane of animal cells, a key stage of carbohydrate metabolism (Mueckler, 1990; Burant et al., 1991; Baldwin, 1993). In mammals, there are at least five distinct sugar transporters (GLUT1–GLUT5). These proteins are expressed in a tissue-specific manner; they exhibit different kinetics, substrate specificity and sensitivity to inhibitors.

Fish genes encoding putative glucose transporters have been identified recently. We have cloned cDNAs from the rainbow trout *Oncorhynchus mykiss* and the common carp *Cyprinus carpio* that are similar to mammalian and avian GLUT1 (Teerijoki et al., 2000; Teerijoki et al., 2001). Planas et al. (Planas et al., 2000) reported molecular identification of the GLUT of the brown trout *Salmo trutta*, which is structurally related to mammalian GLUT4. We and S. Panserat (INRA, France) have cloned the rainbow trout GLUT2 (GenBank AF321816). From an analysis of the derived amino acid sequence, the residues involved in glucose transport (Barrett et al., 1999) appear to be well conserved in these proteins. However, direct evidence for the functionality of fish GLUTs has hitherto been lacking.

The importance of facilitative glucose transporters for fish remains undefined. To study its functional properties, we expressed the putative glucose transporter OnmyGLUT1 in *Xenopus laevis* oocytes. This heterologous expression system has been very useful for characterizing mammalian and protozoan hexose transporters. The relative abundance of OnmyGLUT1 transcripts in rainbow trout embryos suggested that this protein might play an important role in development. In mammals, GLUT1 is referred to as an early isoform because it is expressed at high levels in embryos and foetuses, being gradually substituted for other GLUT types during the course of development (Santalucia et al., 1992; Postic et al., 1994). We have analysed the expression of OnmyGLUT1 at a number of developmental stages (blastula, early and late gastrula, and during somitogenesis and the formation of the vitelline plexus) using whole-mount *in situ* hybridization. We also measured

glucose uptake and determined the ratio of transport to hexokinase activity in embryos and dissociated embryonic cells.

Materials and methods

Studies in Xenopus laevis *oocytes*

Plasmid construction

OnmyGLUT1 was amplified by reverse transcriptase/polymerase chain reaction (RT-PCR) from RNA extracted from newly hatched rainbow trout [*Oncorhynchus mykiss* (Walbaum)] alevins. Primers 5′-ACTGATCAACCACCATG-GATTCAGGCGGCAAGCAAG-3′ (forward) and 5′-TGTG-ATCATTAGAGTTGAGAGTCAGCCCCCAGG-3′ (reverse) were designed to introduce a *Bcl*I restriction site (underlined). The forward primer also included a strong eukaryotic Kozak consensus (CACC). PCR amplification was first carried out with *Pfu* DNA polymerase (Stratagene) (annealing temperature 68 °C). After 20 cycles, *Taq* DNA polymerase (MBI Fermentas) was added to increase the yield of product, and the reaction was continued for an additional 17 cycles (annealing temperature 60 °C). The PCR product was cloned into pcDNA3.1/V5/His-TOPO vector (Invitrogen). The insert was excised by *Bcl*I digestion and cloned into the *Bgl*II site of pSPGT1. This vector contains 5′- and 3′-untranslated sequences of *Xenopus* β-globin mRNA (Kayano et al., 1990). The resulting construct was verified by DNA sequence analysis.

Synthesis of OnmyGLUT1 mRNA and hexose transport

Xenopus laevis oocytes were prepared and used for hexose uptake analyses as described elsewhere (Penny et al., 1998). In brief, mRNA encoding OnmyGLUT1 was transcribed (MEGAscript SP6 with cap-analog; Ambion, Austin, TX, USA) from template linearised with *Xba*I (Promega). Oocytes were injected with either mRNA (15 ng) or RNase-free water. All studies were carried out 24–72 h after microinjection. Uptake of labelled sugars was measured under zero-*trans* conditions. Groups of 8–10 oocytes were placed into 600 µl of Barth's medium containing labelled hexose (0.5 µCi; 18.5 kBq). The reaction was terminated after 20 min, and the oocytes were washed three times with ice-cold Barth's medium. Oocytes were then placed individually into scintillation counter vials. Uptakes were corrected for the uptake by water-injected controls. To verify the functionality of the transporter, D-[U-^{14}C]glucose, 3-*O*-methyl-D-[U-^{14}C]glucose (3-OMG) and D-[U-^{14}C]fructose (all from Amersham Pharmacia Biotech, Amersham, UK) were used. To study kinetics, substrate selectivity and sensitivity to inhibitors, the uptake of labelled D-glucose was measured. Concentrations of D-glucose, competitors and inhibitors (all from Sigma) are given in the figures and Table 1. To measure transport in Na^+-free medium, Na^+ was replaced with equimolar choline chloride. Kinetic parameters were estimated by nonlinear regression analysis using a Michaelis–Menten model, and K_i was calculated using a one-site competition model (PRISM Ver.2, GraphPad, San Diego, CA, USA).

Studies in rainbow trout embryos

Material analysed

Rainbow trout eggs were incubated at 10 °C. The time of formation of one somite pair, τ_s unit (Gorodilov, 1996), was used to determine the developmental age of embryos. At 10 °C, τ_s is equal to 167 min. Samples were collected at $25\tau_s$ (blastula), $36\tau_s$ (early gastrula), 42 and $50\tau_s$ (mid and late gastrula), 60–$62\tau_s$ (5–7 somites), 69–$70\tau_s$ (14–15 somites), 88–$90\tau_s$ (33–35 somites), 115–$117\tau_s$ (60–62 somites), $130\tau_s$ (early vitelline plexus) and $150\tau_s$ (middle vitelline plexus). These developmental stages have been described in detail elsewhere (Gorodilov, 1989; Gorodilov, 1996). At every stage, expression of OnmyGLUT1 was confirmed by RT-PCR. Cytochalasin-B-sensitive uptake of labelled 3-OMG (NEN Life Science Products Boston, MA, USA) was measured as described elsewhere (Teerijoki et al., 2000).

In situ *hybridization*

OnmyGLUT1 in pcDNA3.1-TOPO backbone (Invitrogen) was linearised with *Kpn*I (MBI Fermentas) and used to generate the RNA probe. *In vitro* transcription with T7 RNA polymerase (MBI Fermentas) was carried out in the presence of DIG-oxigenin-11-UTP (Boehringer Mannheim). Embryos were fixed and processed as described previously (Joly et al., 1993) and the transcript was detected using a Boehringer Mannheim kit according to the manufacturer's recommendations. Stained embryos mounted in glycerol were observed and photographed using an Olympus SZX9 stereomicroscope.

Hexose transport

Transport measurements were performed in dissociated embryonic cells using 2-deoxy-D-glucose (2-DOG); this hexose is phosphorylated by hexokinase. Embryos were excised from chorions and washed with phosphate-buffered saline (PBS) to remove yolk. To facilitate dissociation, embryos were incubated in calcium- and magnesium-free PBS for 30 min. Embryos were triturated first with a glass Pasteur pipette and then with an automatic pipette equipped with a 1 ml plastic tip. Cells were filtered through tissue paper, centrifuged for 30 s at 600 ***g*** and resuspended in PBS. Using the exclusion of erythrosin B as an indicator, over 95 % of the cells were found to be alive. Uptake was initiated by adding incubation medium (100 µl), which included PBS, 5 mmol l^{-1} 2-DOG and 1.0 µCi (37.0 kBq) of label (2-deoxy-D-[1-^{3}H]glucose; Amersham Pharmacia Biotech). To account for carrier-independent binding of label, cytochalasin B (50 µmol l^{-1}) was used in parallel incubations. Cells were incubated with labelled 2-DOG for 20 min with periodic shaking. To complete the reaction, the cells were loaded onto the surface of oil (1-bromododecane; Sigma) and centrifuged briefly at 13 000 ***g***. The medium was removed by aspiration, and the oil surface was washed three times with PBS. After removal of the oil, the cells were lysed in water with 0.2 % Triton X-100 and heated to 90 °C for 3 min. To measure total and non-phosphorylated 2-DOG, lysates were divided into two samples.

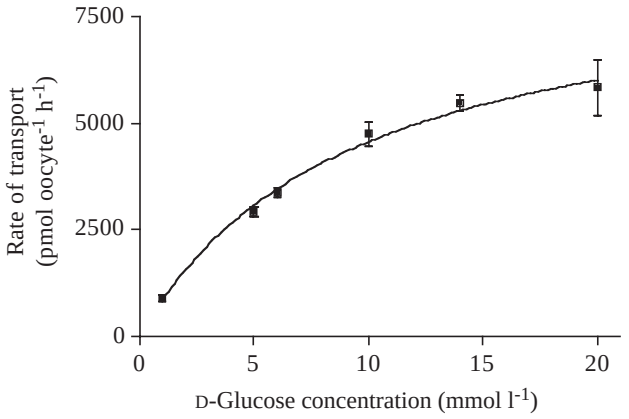

Fig. 1. The kinetics of D-glucose uptake by *Xenopus leavis* oocytes microinjected with OnmyGLUT1 mRNA was studied in three experiments. The results of a typical experiment are presented. Transport rates were determined under zero-*trans* conditions, as described in Materials and methods. Values are means ± S.E.M. (N=8). In this experiment, K_m=9.3±2.3 mmol l^{-1}) and V_{max}=8.8± 1.0 pmol oocyte^{-1} h^{-1}.

Phosphorylated sugar was removed using treatment with $Ba(OH)_2$ and $Zn(SO)_4$ (as described by Colville et al., 1993). Equal volumes of water were added to the parallel samples. Protein was determined using a BioRad kit (catalog no. 500–01210).

Hexokinase assay

Embryos were homogenized in 5 mmol l^{-1} K_2HPO_4, 5 mmol l^{-1} KH_2PO_4 (pH 7.8 at 20 °C), 0.5 mmol l^{-1} EDTA, 250 mmol l^{-1} sucrose and centrifuged at 900 ***g*** for 2 min to remove cell debris. The homogenate was added to a mixture containing 75 mmol l^{-1} Tris (pH 7.4), 7.5 mmol l^{-1} $MgCl_2$, 0.8 mmol l^{-1} EDTA, 1.5 mmol l^{-1} KCl, 0.4 mmol l^{-1} NADP, 2.5 mmol l^{-1} ATP and 0.7 i.u. ml^{-1} glucose-6-phosphate dehydrogenase. The reaction was started by adding glucose to a final concentration of 1 mmol l^{-1}. Increases in light absorbance were measured at 340 nm.

Results

Expression of OnmyGLUT1 in Xenopus laevis *oocytes*

Carrier-mediated transport of D-glucose was examined over a period of 3 days after microinjection of OnmyGLUT1 mRNA. The rate of D-glucose uptake was 2.3 times greater than that of 3-OMG and 13.9 times greater than that of D-fructose. Accumulation of labelled D-glucose in oocytes was linear over a period of 30 min at room temperature (20–22 °C). Kinetic analyses and studies with competitors and inhibitors were carried out 1 or 2 days after microinjections, and oocytes were incubated with D-glucose for 20 min. The kinetic characteristics of D-glucose transport under zero-*trans* conditions were determined in three independent experiments. As expected, transport was saturable and followed Michaelis–Menten kinetics: K_m ranged from 8.3 to 14.9 mmol l^{-1}. Fig. 1 shows the result of a typical experiment. Transport of D-glucose was inhibited by cytochalasin B (1–100 µmol l^{-1}) and phloretin (10–150 µmol l^{-1}) (Fig. 2). The inhibitory effect of phloridzin was detected at concentrations over 500 µmol l^{-1}, and there was no decrease in the rate of transport in Na^+-free medium. K_i for cytochalasin B determined in four experiments

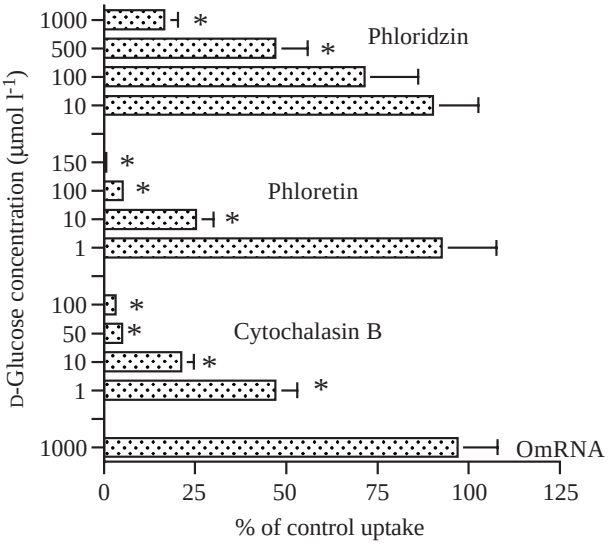

Fig. 2. Inhibition of D-glucose uptake by *Xenopus laevis* oocytes microinjected with OnmyGLUT1 mRNA. Results are expressed as a percentage of uptake of substrate into oocytes incubated without inhibitor (OmRNA). Values are means + S.E.M. (N=8). The results were corrected for uptake into oocytes injected with RNase-free water. *Significantly different from the control value (t-test; P<0.01).

Table 1. *Effect of sugars on D-glucose uptake in OnmyGLUT1-mRNA-injected* Xenopus laevis *oocytes*

Sugar	Uptake (% of control)	Significance
3-OMG	77.4±4.6	P<0.05
1-DOG	74.3±5.8	P<0.05
2-DOG	18.4±2.4	P<0.001
3-DOG	65.6±5.1	P<0.01
6-DOG	60.9±5.9	P<0.001
D-Mannose	19.2±1.2	P<0.001
D-Fructose	96.6±5.5	NS
D-Galactose	92.8±6.8	NS
L-Sorbitol	89.9±4.9	NS
L-Glucose	84.5±7.6	NS
D-Xylose	87.2±6	NS
D-Ribose	86.9±6.2	NS
D-Allose	78.3±6.6	NS
Mannitol	87.8±5.5	NS

Incubation media contained D-glucose (50 µmol l^{-1}) and the sugars indicated at a concentration of 10 mmol l^{-1}.

Values are means ± S.E.M. (N=22).

NS, not significant (t-test).

3-OMG, 3-*O*-methyl-D-glucose; DOG, deoxy-D-glucose.

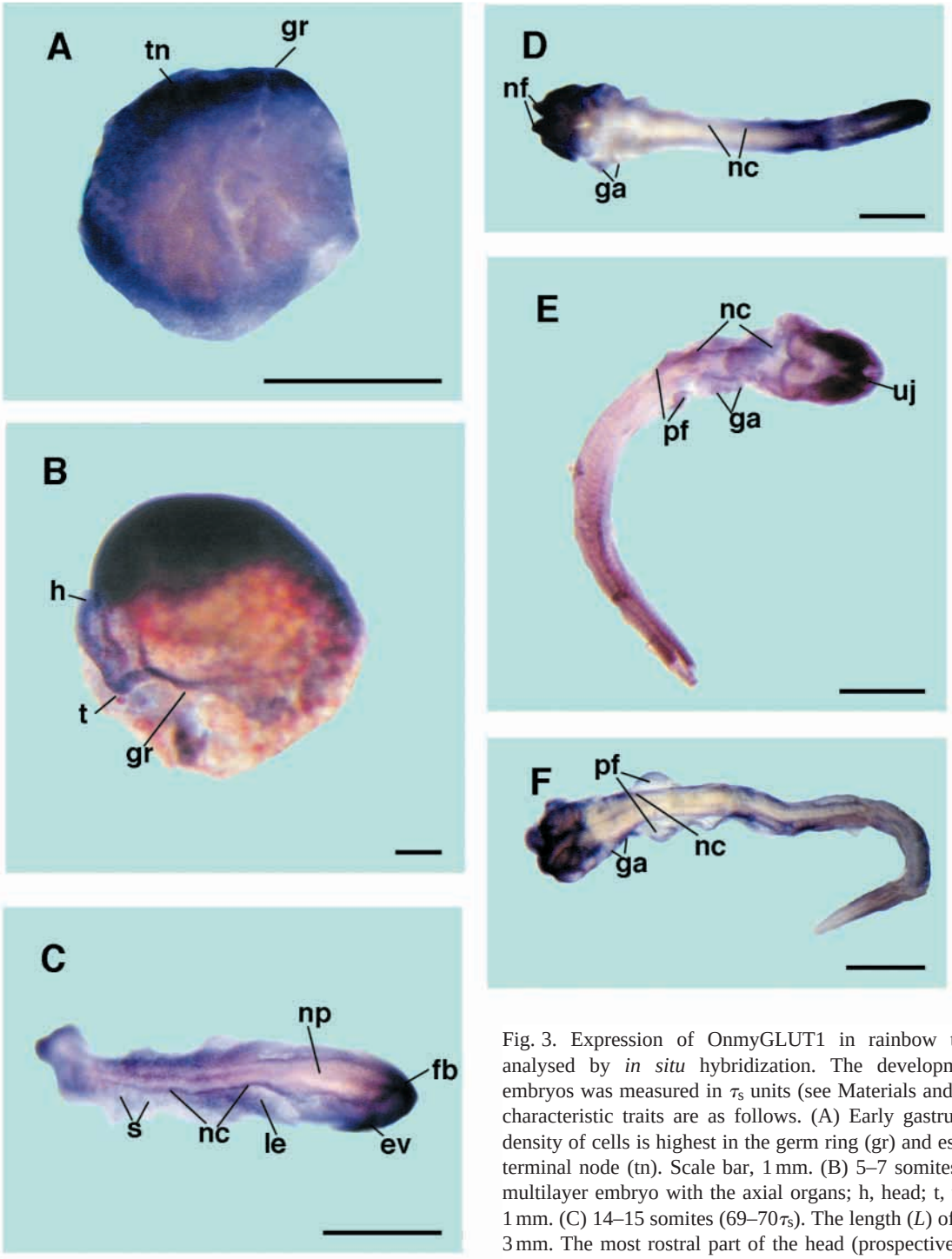

Fig. 3. Expression of OnmyGLUT1 in rainbow trout embryos analysed by *in situ* hybridization. The developmental age of embryos was measured in τ_s units (see Materials and methods); the characteristic traits are as follows. (A) Early gastrula ($36\tau_s$). The density of cells is highest in the germ ring (gr) and especially in the terminal node (tn). Scale bar, 1 mm. (B) 5–7 somites ($60–62\tau_s$). A multilayer embryo with the axial organs; h, head; t, tail. Scale bar, 1 mm. (C) 14–15 somites ($69–70\tau_s$). The length (L) of the embryo is 3 mm. The most rostral part of the head (prospective forebrain, fb) expands as a result of the formation of two eye vesicles (ev). The neural crest (nc) is disposed along the borders separating the neural plate (np) from the lateral ectoderm (le). s, somite. Scale bar, 1 mm. (D) 60–62 somites ($115–117\tau_s$). In these embryos (L=5.8–6.0 mm), the heart contracts and blood circulates in the dorsal aorta; the neural folds (nf) and anlagens of gill arches (ga) are visible. Scale bar, 10 mm. (E) Early vitelline plexus ($130\tau_s$). Somitogenesis has finished, and the development of the cerebral and caudal arteries is in progress; L=7.0–7.2 mm. The upper jaw (uj), gill arches and pectoral fins (pf) are evident. Scale bar, 10 mm. (F) Middle vitelline plexus ($150\tau_s$). Bile fills the gut, and the semicircular canals in the otic vesicles have begun to develop; L=8.5–9.0 mm.

ranged from 1.4 to 2.3 μmol l^{-1} (data not shown). The rate of D-glucose transport decreased in the presence of 3-OMG, 1-deoxy-D-glucose (1-DOG), 2-DOG, 3-DOG, 6-DOG and D-mannose, whereas L-glucose, D-fructose, D-galactose, D-xylose, D-ribose, D-allose, L-sorbitol and mannitol did not compete with D-glucose (Table 1).

OnmyGLUT1 expression in rainbow trout embryos

Expression of OnmyGLUT1 was confirmed at all analysed stages using RT-PCR. Spatial patterns of OnmyGLUT1 expression were analysed using whole-mount *in situ* hybridization. The distribution of transcripts was ubiquitous at early developmental stages. In the early gastrula, OnmyGLUT1 was expressed throughout the embryo; however, hybridization of the probe was most evident on the edges of the blastodisc in the regions of the germ ring and terminal node (Fig. 3A). These structures are formed at the beginning of gastrulation and they are the places with the highest concentration of cells. In the mid and late gastrula, OnmyGLUT1 expression was also clearly evident in all cells (data not shown).

Gastrulation in salmonid fish is completed when the dense core that includes the anlagen of the axial organ complex develops in the middle of the embryonic shield; this consists of the notochord, two bands of unsegmented prospective somite mesoderm and the neural plate (Gorodilov, 1989; Gorodilov, 1996). During the course of somitogenesis, 70–72 somite pairs and primordia of almost all organs and functional systems are formed in rainbow trout embryos. At early somitogenesis (5–7 somites), as well as in the preceding developmental stages, OnmyGLUT1 expression was observed throughout the embryo and blastoderm (Fig. 3B). The spatial pattern of expression gradually became increasingly restricted over the course of development. Uneven distribution of the probe was seen first at the 14–15 somite stage. An abundance of transcript was observed in the forebrain, especially in the region of the eye vesicles (Fig. 3C). OnmyGLUT1 was also expressed in the neural crest. This structure lies at the borders between the neural plate and lateral ectoderm along the entire axis of the embryo. Expression in the neural crest was observed during the whole study period (Fig. 3C–F); however, after the completion of somitogenesis, hybridization of the probe was most apparent in the head part of embryo (Fig. 3E,F). In addition to the neural crest, OnmyGLUT1 expression was found in a number of other embryonic structures such as the gill arches, pectoral fins, upper jaw, primordia of mouth lips and olfactory organs. No hybridization was observed on the dorsal surface of the brain or in the cranial dorsal ectoderm. Diffuse staining of ectoderm was found below the border of the neural crest and in the ventral part of caudal segments. Over the course of development, somitic expression of OnmyGLUT1 decreased along the rostro-caudal axis. At the 60–62 somite stage, hybridization was weak in the anterior trunk myotomes, in contrast to hybridization in the posterior trunk and tail somites (Fig. 3D). No expression of OnmyGLUT1 was evident in the somites of advanced embryos (Fig. 3F). We did not detect the transcript in the notochord, kidney, heart, liver or gut.

Hexose transport activity

Cytochalasin B (50 μmol l^{-1}) significantly decreased 3-OMG uptake by rainbow trout embryos at all analysed stages except the blastula (data not shown). To assess the contribution of transport by OnmyGLUT1 to hexose uptake, we carried out assays with 2-DOG (Table 2). Hexose absorbed by dissociated embryonic cells was separated into phosphorylated and non-phosphorylated moieties, as described in Materials and Methods. Cytochalasin B decreased the content of 2-DOG phosphate (by 75.6 %) in cells to a greater extent than that of non-phosphorylated 2-DOG (by 18.1 %). This result suggested that transport might be rate-limiting for the delivery of glucose to embryonic fish cells. If the rate of uptake were limited by the rate of phosphorylation, inhibitors of transport (such as cytochalasin B) would have affected the accumulation of 2-DOG to a much greater extent than the accumulation of phosphorylated hexose. Hexokinase activity in rainbow trout embryos (1.127±0.037 nmol min^{-1} μg^{-1} protein, mean ± S.E.M., N=8) was two orders of magnitude greater than that of hexose transport and was therefore not rate-limiting in our experiments.

Table 2. *Uptake of 2-DOG by dissociated rainbow trout embryo cells*

	Uptake (pmol μg^{-1} protein)	
	Without cytochalasin B	With cytochalasin B
Total	17.4±0.8*	10.0±0.5
Non-phosphorylated	10.0±0	8.2±0.7

Values are means ± S.E.M. (N=6).
2-DOG, 2-deoxy-glucose.
*Significantly different (t-test, P<0.01).

Discussion

Three putative facilitative glucose transporters have been identified in salmonid fish, although none of these has been characterized functionally. To obtain direct evidence that one of these transporters, OnmyGLUT1, mediates glucose uptake, we expressed the protein in *Xenopus laevis* oocytes. This expression system is commonly used for studies of the functional properties of facilitative glucose transporters because the oocyte has low levels of endogenous glucose transport and efficiently translates foreign RNA. Our results showed that OnmyGLUT1 encodes a fully functional Na^+-independent glucose transporter. The K_m for D-glucose transport was similar to those reported in fish cells. Soengas and Moon (Soengas and Moon, 1995) measured a K_m of 10.4 mmol l^{-1} for 3-OMG uptake by red blood cells of the American eel (*Anguilla rostrata*). The glucose carrier was not identified in that study. However, transport was probably mediated by a GLUT1-like carrier because mammalian erythrocytes harbour exclusively this isoform. We studied 3-OMG uptake in the carp EPC cell line and estimated that the K_m was 8.5 mmol l^{-1} (Teerijoki et al., 2001). A glucose transporter cloned from these cells has been identified as GLUT1. In human cells that employ GLUT1, the K_m for D-glucose ranged from 4 to 10 mmol l^{-1} (Stein, 1990). Similar values have been determined for mammalian GLUT1

expressed in *Xenopus laevis* oocytes. The K_m of rat GLUT1 for 2-DOG is 6.9 mmol l^{-1} (Burant and Bell, 1992). Woodrow et al. (Woodrow et al., 2000) reported a slightly higher affinity of mammalian GLUT1 for D-glucose (K_m=2.5 mmol l^{-1}). D-Glucose transport mediated by OnmyGLUT1 is inhibited by cytochalasin B and phloretin, the classic inhibitors of facilitative glucose transporters. The K_i for cytochalasin B was similar to that of human GLUT1 expressed in *Xenopus laevis* oocytes (Woodrow et al., 2000).

Competitive inhibition analyses (Table 1) were designed to address the substrate specificity of OnmyGLUT1. Uptake of D-glucose was not inhibited by L-glucose, which suggested stereospecificity of transport. Transport activity for D-glucose decreased in the presence of 1-DOG, 2-DOG, 3-DOG and 6-DOG, suggesting that the removal of hydroxyl groups from these positions did not eliminate the ability to compete with D-glucose. D-Mannose, the C2 epimer of D-glucose, also decreased D-glucose transport, suggesting an insignificant role of equatorial hydroxyl in C2. Substitution or removal of the C3 hydroxyl group reduced transport activity. For example, OnmyGLUT1 showed higher activity with D-glucose than with 3-OMG. The inhibitory effects of 2-DOG and mannose were greater than those of 3-DOG and 3-OMG. D-Fructose, D-galactose, D-xylose, D-ribose, D-allose, L-sorbitol and mannitol did not compete with D-glucose. Similar results were obtained with human (Gould et al., 1991; Woodrow et al., 2000) and carp (Teerijoki et al., 2000; Teerijoki et al., 2001) GLUT1. Of all the vertebrate facilitative glucose transporters, OnmyGLUT1 is most similar to GLUT1, both from an analysis of its primary sequence and from its functional characteristics. Mammalian GLUT3 and GLUT4 are characterised by a higher affinity for D-glucose. The K_m of GLUT2 for D-glucose is greater than that of OnmyGLUT1, and GLUT2 is capable of fructose transport.

We analysed hexose transport activity and expression of OnmyGLUT1 in embryos to investigate its importance for rainbow trout development. Uptake of 3-OMG by embryos was inhibited by cytochalasin B. We carried out assays with 2-DOG to determine the relative contributions of transport and phosphorylation to hexose uptake in embryonic cells. Accumulation of 2-DOG-phosphate was almost completely abolished by cytochalasin B, suggesting that metabolized sugar was absorbed predominantly *via* the facilitative carrier.

We studied the temporal and spatial patterns of OnmyGLUT1 expression in rainbow trout embryos. In mammals, expression of GLUT1 is markedly increased after fertilization. Both mRNA and GLUT1 protein are accumulated during pre-implantation in accord with an increase in glucose transport activity during development (Hogan et al., 1991; Morita et al., 1992; Dan-Goor et al., 1997). Using RT-PCR, the earliest stage at which OnmyGLUT1 expression was detected was the blastula. *In situ* hybridization analyses suggested that, at early developmental stages, OnmyGLUT1 was expressed in all cells. Transcripts were found throughout the embryo until the 5–7 somite stage (Fig. 3B), but hybridization was most evident in the germ ring and embryo proper. These structures are known for their high density of cells and active morphogenetic processes. Restricted spatial patterns of OnmyGLUT1 expression were observed beginning from the 14–15 somite stage (Fig. 3C). In older embryos, expression declined during development along the rostro-caudal axis and was undetectable in the myotomes, which are the derivatives of the somites. Expression of mammalian GLUT1 is also reduced during the differentiation of skeletal muscle. Myogenic differentiation includes the fusion of myoblasts into multinuclear myotubes, with subsequent upregulation of muscle-specific protein expression. Guillet-Deniau et al. (Guillet-Deniau et al., 1994) reported the expression of different GLUT proteins at these stages; GLUT1 was abundant in myoblasts, expression of GLUT3 increased markedly at cell fusion and GLUT4 was found exclusively in myotubes.

Expression of OnmyGLUT1 was stable in embryonic neural tissues of the rainbow trout. The transcripts were abundant at the boundary between the neural plate and lateral ectoderm (Fig. 3C–F). In vertebrate embryos, this area harbours the cells of the neural crest (Artinger et al., 1999), which is an exceptionally important structure found exclusively in vertebrates (Baker and Bronner-Fraser, 1997; Gorodilov, 2000). The neural crest gives rise to the connective, skeletal and muscle tissues of the head; moreover, it produces skeleton, pigmented cells and nervous roots in the trunk/tail (Gans and Northcutt, 1983; Langille and Hall, 1989; Couly et al., 1993; Kontges and Lumsden, 1996). The detection of OnmyGLUT1 transcripts in putative derivatives of the neural crest (cartilaginous tissue of the pectoral fins, gill arches and upper jaw) confirmed that OnmyGLUT1 expression is likely to distinguish this cell population. Smoak and Branch (Smoak and Branch, 2000) reported a preponderance of GLUT1 protein in the heart and neural tube in mouse embryos during early organogenesis. An increase in GLUT1 expression in response to hypoglycaemia demonstrated the importance of the enzyme for the embryonic heart. We did not detect OnmyGLUT1 transcripts in the heart of rainbow trout embryos. This finding was unexpected since, in adult fish, this gene is expressed predominantly in the cardiac muscle (Teerijoki et al., 2000). Mammalian GLUT1 is expressed in virtually all embryonic and foetal tissues. In contrast, no OnmyGLUT1 transcripts were found in the notochord, kidney, liver and gut of the rainbow trout embryos.

The distribution of OnmyGLUT1 transcripts in rainbow trout embryos was clearly related to active morphogenetic processes. Expression was most stable in the neural crest cells, which are unique in their totipotent characteristics and extensive migration abilities. It is likely that demand for glucose is increased in differentiating cells because of high metabolic activity. Another possibility is that GLUT is substituted for other isoforms upon differentiation. Recent identification of salmonid GLUT4 and GLUT2 will allow this issue to be addressed.

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References

Artinger, K. B., Chitnis, A. B., Mercola, M. and Driever, W. (1999). Zebrafish *narrowminded* suggests a genetic link between formation of neural crest and primary sensory neurons. *Development* **126**, 3969–3979.

Baker, C. V. H. and Bronner-Fraser, M. (1997). The origins of the neural crest. II. An evolutionary perspective. *Mech. Dev.* **69**, 13–29.

Baldwin, S. A. (1993). Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. *Biochim. Biophys. Acta* **1154**, 18–49.

Barrett, M. P., Walmsley, A. R. and Gould, G. W. (1999). Structure and function of facilitative sugar transporters. *Curr. Opin. Cell Biol*. **11**, 496–502.

Burant, C. F. and Bell, G. I. (1992). Mammalian facilitative glucose transporters: evidence for similar substrate recognition sites in functionally monomeric proteins. *Biochemistry* **31**, 10414–10420.

Burant, C. F., Sivitz, W. I., Fukumoto, H., Kayano, T., Nagamatsu, S., Seino, S., Pessin, J. E. and Bell, G. I. (1991). Mammalian glucose transporters: structure and molecular regulation. *Recent Progr. Horm. Res.* **47**, 349–388.

Colville, C. A., Seatter, M. J., Gould, G. W. and Thomas, H. M. (1993). Kinetic analysis of the liver-type (GLUT2) and brain-type (GLUT3) glucose transporters in *Xenopus* oocytes: substrate specificities and effects of transport inhibitors. *Biochem. J.* **290**, 701–706.

Couly, G. F., Coltey, P. M. and Le Douarin, N. M. (1993). The triple origin of skull in higher vertebrates: a study in quail–chick chimeras. *Development* **117**, 409–429.

Dan-Goor, M., Sasson, S., Davarashvili, A. and Almagor, M. (1997). Expression of glucose transporter and glucose uptake in human oocytes and preimplantation embryos. *Human Reprod.* **12**, 2508–2510.

Gans, C. and Northcutt, R. G. (1983). Neural crest and origin of vertebrates: a new head. *Science* **220**, 268–274.

Gorodilov, Y. N. (1989). Comparative analysis of the dynamics of early ontogeny of species of genus *Salmo*. *J. Ichtyol.* **29**, 19–31.

Gorodilov, Y. N. (1996). Description of the early ontogeny of the Atlantic salmon, *Salmo salar*, with a novel system of interval (state) identification. *Env. Biol. Fish.* **47**, 109–127.

Gorodilov, Y. N. (2000). The fate of Spemann's organizer. *Zool. Sci.* **17**, 1197–1220.

Gould, G. W., Thomas, H. M., Jess, T. J. and Bell, G. I. (1991). Expression of human glucose transporters in *Xenopus* oocytes: kinetic characterization and substrate specificities of the erythrocyte, liver and brain isoforms. *Biochemistry* **30**, 5139–5145.

Guillet-Deniau, I., Leturque, A. and Girard, J. (1994). Expression and cellular localization of glucose transporters (GLUT1, GLUT3, GLUT4) during differentiation of myogenic cells isolated from rat foetuses. *J. Cell Sci.* **107**, 487–496.

Hogan, A., Heyner, S., Charron, M. J., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Thorens, B. and Schultz, G. A. (1991). Glucose transporter gene expression in early mouse embryos. *Development* **113**, 363–372.

Joly, J. S., Joly, C., Schulte-Merker, S., Boulekbache, H. and Condamine, H. (1993). The ventral and posterior expression of the even-skipped homeobox gene eve 1 is perturbed in dorsalized and mutant embryos. *Development* **119**, 1261–1275.

Kayano, T., Burant, C. F., Fukumoto, H., Gould, G. W., Fan, Y. S., Eddy, R. L., Byers, M. G., Shows, T. B., Seino, S. and Bell, G. I. (1990). Human facilitative glucose transporters. Isolation, functional characterization and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). *J. Biol. Chem.* **265**, 13276–13282.

Köntges, G. and Lumsden, A. (1996). Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. *Development* **122**, 3229–3242.

Langille, R. M. and Hall, B. K. (1989). Developmental processes, developmental sequences and early vertebrate phylogeny. *Biol. Rev.* **64**, 73–91.

Morita, Y., Tsutsumi, O., Hosoya, I., Taketani, Y., Oka, Y. and Kato, T. (1992). Expression and possible function of glucose transporter protein GLUT1 during preimplantation mouse development from oocytes to blastocysts. *Biochem. Biophys. Res. Commun.* **188**, 8–15.

Mueckler, M. (1990). Family of glucose-transporter genes. Implications for glucose homeostasis and diabetes. *Diabetes* **39**, 6–11.

Penny, J. I., Hall, S. T., Woodrow, C. J., Cowan, G. M., Gero, A. M. and Krishna, S. (1998). Expression of substrate-specific transporters encoded by *Plasmodium falciparum* in *Xenopus laevis* oocytes. *Mol. Biochem. Parasitol*. **93**, 81–89.

Planas, J. V., Capilla, E. and Gutierrez, J. (2000). Molecular identification of a glucose transporter from fish muscle. *FEBS Lett.* **481**, 266–270.

Postic, C., Leturque, A., Printz, R. L., Maulard, P., Loizeau, M., Granner, D. K. and Girard, J. (1994). Development and regulation of glucose transporter and hexokinase expression in rat. *Am. J. Physiol.* **266**, E548–E559.

Santalucia, T., Camps, M., Castello, A., Munoz, P., Nuel, A., Testar, X., Palacin, M. and Zorzano, A. (1992). Developmental regulation of GLUT-1 (erythroid/Hep G2) and GLUT-4 (muscle/fat) glucose transporter expression in rat heart, skeletal muscle and brown adipose tissue. *Endocrinology* **130**, 837–846.

Smoak, I. W. and Branch, S. (2000). Glut-1 expression and its response to hypoglycemia in the embryonic mouse heart. *Anat. Embryol.* **201**, 327–333.

Soengas, J. L. and Moon, T. W. (1995). Uptake and metabolism of glucose, alanine and lactate by red blood cells of the American eel *Anguilla rostrata*. *J. Exp. Biol.* **198**, 877–888.

Stein, W. D. (1990). *Channels, Carriers and Pumps: An Introduction to Membrane Transport*. San Diego: Academic Press. 137pp.

Teerijoki, H., Krasnov, A., Pitkänen, T. I. and Mölsä, H. (2000). Cloning and characterization of teleost fish rainbow trout (*Oncorhynchus mykiss*) glucose transporter. *Biochim. Biophys. Acta* **1493**, 290–294.

Teerijoki, H., Krasnov, A., Pitkänen, T. I. and Mölsä, H. (2001). Monosaccharide uptake in common carp (*Cyprinus carpio*) EPC cells is mediated by facilitative glucose transporter. *Comp. Biochem. Physiol.* **128**B, 483–491.

Woodrow, C. J., Burchmore, R. J. and Krishna, S. (2000). Hexose permeation pathways in *Plasmodium falciparum*-infected erythrocytes. *Proc. Natl. Acad. Sci. USA* **97**, 9931–9936.