Zebrafish Reveals Different and Conserved Features of Vertebrate Neuroglobin Gene Structure, Expression Pattern, and Ligand Binding*§

Received for publication, February 24, 2004, and in revised form, March 22, 2004.

Published, JBC Papers in Press, March 31, 2004, DOI 10.1074/jbc.M402011200

Christine Fuchs‡§, Valeska Heib‡§, Laurent Kiger‡, Mark Haberkamp§, Anja Roesner‡, Marc Schmidt‡, Djemel Hamdane‡, Michael C. Marden¶, Thomas Hankeln‡, and Thorsten Burmester**

From the ‡Institute of Molecular Genetics, Johannes Gutenberg University of Mainz, D-55099 Mainz, Germany, §Institute of Zoology, Johannes Gutenberg University of Mainz, D-55099 Mainz, Germany, and **INSERM U473, 84 rue du General Leclerc, F-94276 Le Kremlin-Bicetre, France

Neuroglobin has been identified as a respiratory protein that is primarily expressed in the mammalian nervous system. Here we present the first detailed analysis of neuroglobin from a non-mammalian vertebrate, the zebrafish Danio rerio. The zebrafish neuroglobin gene reveals a mammalian-type exon-intron pattern in the coding region (B12.2, E11.0, and G7.0), plus an additional 5'-non-coding exon. Similar to the mammalian neuroglobin, the zebrafish protein displays a hexacoordinate deoxy-binding scheme. Flash photolysis kinetics show the competitive binding on the millisecond timescale of external ligands and the distal histidine, resulting in an oxygen affinity of 1 torr. Western blotting, immune staining, and mRNA in situ hybridization demonstrate neuroglobin expression in the fish central nervous system and the retina but also in the gills. Neurons containing neuroglobin have a widespread distribution in the brain but are also present in the olfactory system. In the fish retina, neuroglobin is mainly present in the inner segments of the photoreceptor cells. In the gills, the chloride cells were identified to express neuroglobin. Neuroglobin appears to be associated with mitochondria-rich cell types and thus oxygen consumption rates, suggesting a myoglobin-like function of this protein in facilitated oxygen diffusion.

Transport and storage of oxygen in vertebrate animals are typically mediated by globins, small proteins that bind O2 by the means of a porphyrin-coordinated Fe2+ ion (1–9). The heterotetrameric hemoglobin is present in red blood cells of nearly all vertebrates and transports O2 in the circulatory system from the respiratory surfaces to the inner organs. The monomeric myoglobin, typically found in the myocytes of cardiac and striated muscles, facilitates intracellular O2 diffusion to the mitochondria and stores O2 (3, 4) but also functions as an nitric-oxide dioxygenase (5). Neuroglobin (Ngb) and cytoglobin are two recently discovered vertebrate globins (6–9). Whereas cytoglobin might play a role in collagen synthesis (10, 11), the leading hypotheses suggest Ngb to be involved in neuronal oxygen homeostasis (6, 12–16).

Ngb shares only a few amino acids with vertebrate hemoglobin and myoglobin (<25% identity) but rather resembles the nerve-specific globins known in some invertebrates (6, 13). In fact, phylogenetic analyses suggest an ancient origin of Ngb, probably diverging from the other globins before the Protostomia-Deuterostomia split (6, 8). Mouse and human deoxy-Ngb display hexacoordinate hemochrome-binding schemes at the Fe2+/5 (17, 18). The proximal histidine can be replaced by an external ligand such as O2, resulting in an oxygen affinity (P50) of ~1–2 torr, similar to that of myoglobin (6, 17).

Initially, Ngb was found to be predominantly expressed in the brains of man and mouse (6), but recent analyses show Ngb to be also present in the peripheral nervous system and some non-neuronal endocrine tissues (19–21). Particularly high amounts of Ngb were observed in the mammalian retina where it could reach a concentration similar to that of myoglobin in muscle cells (14). This study also suggests that, at the subcellular level, Ngb is localized adjacent to the mitochondria. In cultured neuronal cells from the mouse brain, synthesis of Ngb is enhanced under low oxygen conditions (12), although these results could not be confirmed by in vivo studies (22). The presence of Ngb promotes the survival of cultured neuronal cells at low oxygen levels, and overexpression of Ngb may protect neurons from hypoxic-ischemic injury in vivo and in vitro (12, 15).

The available data suggest that Ngb expression levels correlate with high oxygen consumption rates, implying an important role of Ngb in neuronal O2 supply, although other functions of Ngb are still conceivable (13, 23). Whereas Ngb was originally identified in mammalian species (6), it is also present in fishes (24), suggesting a widespread occurrence in vertebrates. The investigation of Ngb function in non-mammalian species is an essential prerequisite for the understanding of its role in the vertebrate metabolism. Here, we have carried out a detailed genetic, biochemical, and expression analysis of Ngb from the zebrafish Danio rerio.

EXPERIMENTAL PROCEDURES

Cloning and Sequencing of D. rerio Ngb cDNA and Gene—D. rerio were kept at 28 °C in a freshwater aquarium. Genomic DNA of D. rerio
was extracted using the Qiagen DNeasy kit. Several oligonucleotide primers were designed according to the Ngb cDNA sequence (24) and partial data base entries of the zebrafish Ngb gene. Overlapping fragments were amplified using the SAWADY "Mid Range" PCR system (PeqLab). The sequences were obtained after the cloning of the PCR products into the pCR4-TOPO vector (Invitrogen). 5'-RACE experiments were carried out with the Invitrogen kit according to the manufacturer's instructions using ~1 μg of D. rerio total RNA and three nested oligonucleotide primers. Sequences were obtained either directly from the PCR products or after cloning into the pCR4-TOPO vector (Invitrogen). Sequences were analyzed by applying the GeneDoc 2.6 (25), MatInspector V2.2 (26), and BLAST searches (27) using Eukaryotic Promoter Data base, release 76 (28).

**Purification of Recombinant Ngb**—The complete D. rerio Ngb cDNA was cloned into the pET3a expression vector (Novagen) using PCR-generated NdeI and BglII restriction sites. The plasmid was transformed into the DH5α (Novagen) using PCR-generated NdeI and BglII restriction sites. 50-watt quartz halogen lamp with an interference filter. The positions of the start and stop codons and the four introns in the Ngb gene. The nucleotide sequence of the zebrafish Ngb cDNA has been determined previously (24). We designed multiple primers that amplified the coding region of the Ngb cDNA. The sequences were determined with the cDNA reveals the presence of four introns. The second, third, and fourth introns are positioned within the coding region of the Ngb gene. The nucleotide sequence of the zebrafish Ngb cDNA has been determined previously (24). We designed multiple primers that amplified the coding region of the Ngb cDNA. The sequences were determined with the cDNA reveals the presence of four introns. The second, third, and fourth introns are positioned within the coding region of the Ngb gene. The nucleotide sequence of the zebrafish Ngb cDNA has been determined previously (24). We designed multiple primers that amplified the coding region of the Ngb cDNA. The sequences were determined with the cDNA reveals the presence of four introns. The second, third, and fourth introns are positioned within the coding region of the Ngb gene. The nucleotide sequence of the zebrafish Ngb cDNA has been determined previously (24).
The Ngb promoter region (Fig. 1). Restrictive silencer elements (NRSEs) were also identified in box are present (Supplemental Table S1). Two putative neuron-binding sites, CCAAT/enhancer-binding protein, and CAAT gene show that the putative promoter lacks a TATA box, whereas multiple other regulatory sequences such as GATA-

Typically, an HRE includes two HIF-1 motifs (in direct or inverted orientation) or one HIF-1 motif combined with an erythropoietin (EPO) box (32) or a HIF ancillary sequence (33). Five such closely spaced motif combinations are present in the zebrafish Ngb gene region (Fig. 1).

Ligand-binding Kinetics of Zebrafish Neuroglobin—The Danio Ngb was expressed using the pET3a vector system. The recombinant protein was purified by ammonium sulfate precipitation followed by ion-exchange and size exclusion chromatography (Supplemental Fig. S2). The ferrous deoxy form of Ngb shows a typical hexacoordinated absorption spectrum (Fig. 2A) as characterized by large amplitudes for the $\alpha$ band (560 nm) and the Soret band (426 nm) (17). The kinetics after CO photodissociation revealed a biphasic form. The rapid phase is competitive binding of CO or the distal histidine residue. For higher CO levels, the rate and relative amplitude of this phase increases. At low CO levels, the histidine binding becomes more competitive and the slow phase becomes more prominent. The slow phase involves the replacement of the histidine by CO and requires several seconds for a complete return to the initial CO-bound state. From the kinetics at various CO levels, one can extract the CO on-rate and the histidine both on-rate and off-rate (Table I). Similarly, competition of oxygen and CO can be used to obtain the rate coefficients for oxygen. Note that two ligand affinities are given in the table. The intrinsic affinity is the ratio of the binding rates for a given ligand. This would be the affinity in the absence of competing ligand. The overall oxygen affinity, taking into account the competing histidine ligand, is calculated to be $-1$ torr, similar to human Ngb without dithiothreitol (34). With dithiothreitol, the disulfide bond is broken and human Ngb shows a lower oxygen affinity. This effect was not observed for Danio Ngb.

Detection of Ngb Protein in Zebrafish Tissues—Specific antibodies against recombinant zebrafish Ngb were raised in rabbits and further purified by affinity chromatography. In Western blotting, these antibodies detect the recombinant Ngb at $-16$ kDa (Fig. 3A). Ngb was also stained by the antibody in extracts from the brain, the total eye, and the gills but not in the muscle or the blood. As already observed in mouse (14), the apparent molecular mass of the native Ngb is slightly higher than that of the recombinant protein. The reason for this discrepancy is still unknown but may be explained by posttranslational modifications. After preadsorption of the antibodies with the antigen, the Ngb band disappeared in Western blots of retinal and brain tissues, demonstrating the specificity of the anti-Ngb antibodies. To investigate whether Ngb may be released from the cells, the total brain was incubated for 2 h in PBS. However, no Ngb could be detected in the supernatant while there was a signal in the brain extracts (Fig. 3B).

Expression Pattern of Danio Ngb by mRNA in Situ Hybridization—To localize the sites of Ngb mRNA expression in zebrafish, frontal cryosections of head regions were analyzed by in situ hybridization (ISH) using an in vitro transcribed antisense RNA probe. Cross-sections from several layers were inspected (Supplemental Fig. S3). A perinuclear signal typical for mRNA hybridization of Ngb was found in the neuronal somata of essentially all of the regions of the Danio brain (Fig. 4) (see also Supplemental Fig. S4 and Supplemental Table S2). Control experiments that included nonspecific RNA (mouse intes-

![Fig. 2. Ligand binding of recombinant D. rerio Ngb. A, absorption spectra equilibrated under CO (solid line) or nitrogen (deoxy, dashed line). B, recombination of CO to zebrafish Ngb at three CO concentrations: 10, 100, and 1000 $\mu$M. The biphasic kinetics show the competition between CO and His binding. As the CO concentration is increased, less His binds and the amplitude of the slow phase decreases.

| Ligand binding parameters to Ngb | pH 7.0, 100 mM phosphate buffer based on flash photolysis results. |
|---------------------------------|-----------------------------------------------------|
| **CO**  | **Oxygen**  | **Histidine**  | **O$_2$** |
| $k_{on}$ | $k_{off}$ | $K_a$ | $K_{d, O_2}$ | $K_{on}$ | $K_{off}$ | $K_{d, His}$ | $P_{50}$ |
|**Danio** | 70 | 250 | 0.3 | 1.3 | 2500 | 2 | 1250 | 0.9 |
| Human Ngb$^a$ | 40 | 140 | 0.8 | 5.7 | 2000 | 7 | 280 | 0.9 |
| Ngb + DTT$^a$ | 50 | 170 | 0.8 | 4.7 | 2000 | 0.6 | 3300 | 8.4 |

$^a$ Hamdane et al. (32).
and the myelencephalon, signals could be seen in the In the rhombencephalon, which includes the metencephalon.

in neuronal populations such as the preglomerular nucleus, putative neurons scattered in the white matter of the brain and Danio of the rhombencephalon. The other parts of the cephalic region of the telencephalon and the involved in visual signal processing, the encephalon. ISH signal was also present in two other regions

zone of the

tuberculum
telencephalic
frontal sections showed the presence of Ngb mRNA in various brain regions (Supplemental Fig. S4 and Supplemental Table S2), e.g. Ngb mRNA was also detected in brain regions of the visual system, predominantly in parts of the tectum opticum and the torus semicircularis, which are both part of the mesencephalon. ISH signal was also present in two other regions involved in visual signal processing, the area dorsalis telen-cephali of the telencephalon and the medulla oblongata, a part of the rhombencephalon. The other parts of the Danio brain in which strong ISH signals were observed included parts of the telencephalic area dorsalis telencephali, the diencephalic posterior tuberculum, the hypothalamus, and the synencephalon. In the rhombencephalon, which includes the metencephalon and the myelencephalon, signals could be seen in the formatio reticularis.

Ngb ISH staining was also noticed in the telencephalon, containing the bulbus olfactorius (Fig. 5A). Intense Ngb ISH signal could be detected in the sensory epithelium of the peripheral olfactory organ. Strong Ngb mRNA labeling was also observed in distinct layers of the zebrafish retinal nerve (Fig. 5B). The outer and inner nuclear layers, which contain the nuclei, and the ganglion cell layer were heavily stained with the Ngb probe, whereas the outer plexiform layer and the outer segments of the photoreceptor cells appeared to be unstained. The head cross-sections revealed that Ngb is also present in the lateral regions of the secondary lamella.

Immunodetection of Danio Ngb Protein—The presence of the Ngb protein in zebrafish tissues was monitored by indirect immunofluorescence experiments. An affinity purified α-Danio Ngb antibody was applied on cryosections from both fixed and unfixed tissues. In agreement with the mRNA ISH data, we observed scattered Ngb immune reactivity of the brain neurons. Particularly strong staining of neurons was observed in the diencephalic lateral hypothalamus (Fig. 6A). In control experiments without first antibody, no staining of the neurons was observed (Fig. 6B). In the retina, no anti-Ngb immune staining was found either in the cells of the retinal pigment epithelium or in the outer segments of the photoreceptors. Bright immune staining was observed in the photoreceptor layer, whereas there was little but detectable immune reaction in the outer and inner plexiform layers and at the ganglion cells (Fig. 6C). No signal was present in the appropriate control experiments (not shown). In the gills, we observed scattered staining of cells that most probably corresponded to the chlo-ride cells of the secondary lamella (Fig. 6D).

**DISCUSSION**

Ngb is a recently identified member of the vertebrate globin superfamily (6) that is probably present in all of the vertebrate taxa including fish (24), amphibians, and birds. Although most experiments support the idea of an important role of Ngb in oxygen homeostasis of nerve tissues (6, 12–16), other func-tions or additional functions of Ngb are still conceivable (13, 16)

2 C. Fuchs, P. Kugel Stadt, T. Hankeln, and T. Burmester, unpublished data.
and many open questions remain to be solved. So far, biochemical and molecular studies on Ngb have been carried out exclusively with mammalian species. However, a comparative approach involving non-mammalian species offers a promising tool for the identification of conserved Ngb features and thus for the further understanding of Ngb function.

Conservation and Differences between Fish and Mammalian Ngb Genes—Ngb is a highly conserved protein with substitution rates that are ~3-fold lower than those in hemoglobin and myoglobin (6, 24, 35). This is also true for the gene structure, which contains three conserved introns in the coding regions of fish and mammalian Ngb genes. While the introns in B12.2 and G7.0 are present in all vertebrate and many invertebrate globin genes (13, 36), the intron in E11.0 is unique to Ngb. The additional short intron in the 5′-untranslated region is most likely homologous to an intron at a similar position in the Tetraodon nigroviridis and Takifugu rubripes genes (data not shown). Such an intron is absent from the mammalian neuroglobins and may have been acquired early in fish evolution before the Ostariophysi (D. rerio) and the Acanthopterygii globins and may have been acquired early in fish evolution (Fig. 1). However, a comparison of the zebrafish Ngb gene with that of T. nigroviridis and T. rubripes does not reveal any positional conservation of these regulatory elements (35).

The actual function of the putative HREs in hypoxia regulation of the zebrafish Ngb genes requires experimental confirmation in future studies. In several mammalian genes, neuron-specific gene expression was mediated by a 21-bp sequence motif, the NRSE that binds to the neuron-restrictive silencer factor (NRSF) (41, 42). Putative NRSE motifs have been identified in mouse and human Ngb genes (43), and two such elements were present in the 5′-promoter region of the Danio Ngb gene (Supplemental Table S1 and Supplemental Fig. S1). Although the regulatory function of these NRSEs remains to be established in both fish and mammalian Ngb, they possibly explain the largely neuron-specific expression of Ngb.

Fish and Mammalian Ngb Are Hexacoordinated Globins with Similar Ligand-binding Kinetics—Similar to the mammalian Ngb (17, 18) and various other animal and plant hemoglobins (44), deoxygenated zebrafish Ngb displayed a hexacoordinated hemochrome-binding scheme at the Fe2+ of the porphyrin ring. The ligand-binding kinetics of zebrafish Ngb are similar to those of human Ngb, displaying the biphasic form for competitive binding of the internal His with the external ligand. The rate coefficients for Danio Ngb are approximately twice those of human Ngb, indicating a faster passage in the heme pocket. Because all of the rates are increased by the same factor, the overall observed affinity does not change significantly. As for mammalian Ngb, the Danio Ngb oxygen affinity is ~1 torr, suggesting that this parameter has been maintained
throughout the evolutionary process. There is a major difference in the dependence on the kinetics on the possible disulfide bond. Although human Ngb oxygen affinity decreased to ~10 torr (34), Danio Ngb showed little effect. Note that the first Cys in Danio was shifted by two positions relative to the human Ngb sequence.

Neuronal Expression of Ngb in the Vertebrate Brain—As in the central nervous system of mammals (6, 19, 21), Ngb was expressed in brain neurons (Figs. 3–6). Because of the instability of the purified anti-Ngb antibody, we did not carry out a thorough immunohistochemical analysis of the fish brain. Nevertheless, in regions inspected by both methods, ISH and immunofluorescence, the observed patterns matched and demonstrated the presence of Ngb mRNA and protein in the somata of most if not all of the nerve cells. No evidence was found for glial expression of Ngb in fish, which corresponds to the data obtained in mammals (19–21). Thus, the neuronal expression of Ngb was conserved in gnathostomian vertebrates for ~420 million years (37). A novel finding of this study was the observation of strong Ngb expression in the sensory part of the receptor cells of the zebrafish olfactory system (Fig. 5A). Significant Ngb was also present in the following parts of the olfactory tract in the telencephalon and the olfactory cortex. Because of the strong calcification of the rodent olfactory system, similar staining experiments have not been carried out in these species. Thus, it remains to be established whether high Ngb expression also occurs in the olfactory region of the mammals or whether this is a unique feature of the teleost fish.

Because the immunofluorescence signal in the brain was only clearly visible at high magnifications, its total concentration in the brain appeared to be low. Nevertheless, the fish retina and the gills showed bright fluorescence and thus may contain higher amounts of Ngb.

High Concentrations of Ngb in the Photoreceptor Inner Segments—The mouse retina has been observed so far to be the highest Ngb-expressing tissue in mouse with the Ngb protein reaching a concentration similar to that of myoglobin in the striated muscle (~100 μM) (14). The ISH pattern of Ngb mRNA in the fish retina was similar to that in mouse with the exception that we observed some ISH signals in the fish inner plexiform layer (Fig. 5B). Although there was, as in mouse, detectable amounts of Ngb protein present in the ganglion cell and the plexiform layers, most Ngb in the zebrafish retina actually appeared to be located in the inner segments of the photoreceptor cells (Fig. 6B). If the idea of an oxygen transport function of Ngb (6, 14, 16) was correct, it may be explained by the high energy requirements of the fish cones and rods. Whereas upon light adaptation, the pupil diameter of the mammalian retina decreased, most fish did not show a similar response (45). Rather, the cone cells of the fish retina displayed light-induced shortening of the myoid, the neck-region located between the ellipsoid and the perinuclear region of the inner segments (46). Consequently, a high concentration of mitochondria in the ellipsoid region has been observed. Thus, the observation of the presence of apparently high Ngb concentrations in the inner segments agrees with previous observation in the mouse retina that suggests an association of Ngb with mitochondria (14).

Wittenberg and Wittenberg (47) observed an extracellular hemoprotein in the choroid blood from perfused retina of two basal teleost fish species (bowfin and bluefish). The hemochrome absorption spectra of these globins were similar to those of mammalian and fish Ngb. Thus, we speculated that this heme protein may correspond to Ngb (14, 24). However, neither the Western blotting experiments (Fig. 3) nor the immunofluorescence data (Fig. 6) provided any evidence that Ngb may be found in the blood or the extracellular space. Therefore, it remains uncertain whether the retinal heme protein identified in the bowfin and the bluefish (47) corresponds to Ngb and, even if it does, whether it represents as proposed by those authors an intracellular globin that has been artificially released into the blood during the preparation procedure.

Ngb in the Chloride Cells Suggests Another Association of Ngb with Mitochondria—With the exception of the expression in some endocrine tissues (19, 20), Ngb has been assumed to be an exclusively neuronal protein. Here we show by immunofluorescence and Western blotting that Ngb was also present in the chloride cells of zebrafish gills. Fish gills perform a variety of physiological functions including respiratory gas exchange, ion exchange, and excretion (48, 49) and are considered as high energy-demanding tissues (50, 51). In freshwater fish, the chloride cells are the principal site of Ca^{2+}, Cl^{−}, and possibly also Na^{+} uptake (52) and are known to be rich in mitochondria (52, 53). Again, this observation suggests an association of Ngb with the mitochondria (14) and is in line with the hypothesis that Ngb is involved in cellular oxygen consumption.

Implications for Vertebrate Ngb Function—The phylogenetic origin of Ngb dates back to before the time when the Protostomia and Deuterostomia diverged, suggesting a conserved function of this protein in the metabolism of the animal (6, 13, 14, 24). The Ngb gene structure, oxygen-binding kinetics, and expression patterns are globally similar in mammals and fish. Differences in the retinal expression pattern as well as the presence of Ngb in the fish gills may be easily explained by different oxygen requirements within these tissues. It should be stressed that, similar to mammals, fish Ngb concentration appears to be correlated with an abundant presence of mitochondria in the cells, although there is no evidence that Ngb is located within these organelles. Therefore, our results support the hypothesis of an important role of Ngb in oxygen-linked metabolism (14, 16). Whether Ngb has a myoglobin-like function in supplying oxygen to the respiratory chain by facilitated diffusion from the cell membrane to the mitochondria (3, 4) or whether it actually sustains the energy metabolism of the cell via another, still unknown mechanism (13, 16), remains to be established.

Acknowledgments—We thank S. Reuss and G. Technau for help with the microphotography, M. Schaffeld and U. Wolfrum for discussions, and J. Markl for excellent working facilities.

REFERENCES
1. Dickerson, R. E., and Geis, I. (1983) Hemoglobin: Structure, Function, Evolution, and Pathology, Benjamin/Cummings Publications Co., Menlo Park, CA
2. Wittenberg, J. B. (1992) Adv. Comp. Environ. Physiol. 13, 60–85
3. Wittenberg, J. B., and Wittenberg, B. A. (2003) J Exp. Biol. 206, 2011–2020
4. Merx, M. W., Flögel, U., Stumpe, T., Godecke, A., Decking, U. K., and Schrader, J. (2002) FASEB J. 15, 1071–1079
5. Flögel, U., Merx, M. W., Godecke, A., Decking, U. K., and Schrader, J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 735–740
6. Burmester, T., Weich, B., Reinhardt, S., and Hankeln, T. (2000) Nature 407, 520–523
7. Kawada, N., Kristensen, D. B., Asahina, K., Nakatani, K., Minamiyama, Y., Seki, S., and Yoshizato, K. (2001) J. Biol. Chem. 276, 25318–25323
8. Burmester, T., Ebner, B., Weich, B., and Hankeln, T. (2002) Mol. Biol. Evol. 19, 416–421
9. Trent, J. T., III, Watts, R. A., and Hargrove, M. S. (2001) J. Biol. Chem. 276, 30106–30110
10. Nakatani, K., Okuyama, H., Shimahara, Y., Sasaki, S., Kim, D. H., Nakajima, Y., Seki, S., Kawada, N., and Yoshizato, K. (2004) Lab. Invest. 84, 91–101
11. Schmidt, M., Gerlach, F., Avivi, A., Laufs, T., Wystub, S., Simpson, J. C., Nevo, E., Sailer-Reinhardt, S., Reuss, S., Hankeln, T., and Burmester, T. (2004) J. Biol. Chem. 279, 8063–8069
12. Sun, Y., Jin, K., Mao, X. O., Zhu, Y., and Greenberg, D. A. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 15309–15311
13. Pesce, A., Bolognesi, M., Ascenzi, P., Bocedi, A., Dewilde, S., Moens, L., Hankeln, T., and Burmester, T. (2002) EMBO Rep. 3, 1146–1151
14. Schmidt, M., Geijl, A., Laufs, T., Hankeln, T., Wolfrum, U., and Burmester, T. (2003) J. Biol. Chem. 278, 1912–1915
15. Sun, Y., Jin, K., Peel, A., Mao, X. O., Xie, L., and Greenberg, D. A. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 3497–3500

Acknowledgments—We thank S. Reuss and G. Technau for help with the microphotography, M. Schaffeld and U. Wolfrum for discussions, and J. Markl for excellent working facilities.
24122

Zebrafish Neuroglobin

16. Burmester, T., and Hankeln, T. (2004) Neur. Physiol. Sci., in press
17. Dewilde, S., Kiger, L., Burmester, T., Hankeln, T., Baudin-Creuza, Y., Aerts, T., Marden, M. C., Caubergs, R., and Moens, L. (2001) J. Biol. Chem. 276, 38949–38955
18. Trent, J. T., III, and Hargrove, M. S. (2002) J. Biol. Chem. 277, 19538–19545
19. Reuss, S., Saaler-Reinhardt, S., Weich, B., Wystub, S., Reuss, M., Burmester, T., and Hankeln, T. (2002) Neuroscience 115, 645–656
20. Reuss, S., Saaler-Reinhardt, S., Weich, B., Wystub, S., Reuss, M., Burmester, T., and Hankeln, T. (2003) Anal. Biochem. 319, 2292–2298
21. Dewilde, S., Kiger, L., Burmester, T., Hankeln, T., Baudin-Creuza, Y., Aerts, T., Marden, M. C., Caubergs, R., and Moens, L. (2001) J. Biol. Chem. 276, 38949–38955
22. Altschul, S. F., Madden, T. L., Scha...
Zebrafish Reveals Different and Conserved Features of Vertebrate Neuroglobin Gene Structure, Expression Pattern, and Ligand Binding
Christine Fuchs, Valeska Heib, Laurent Kiger, Mark Haberkamp, Anja Roesner, Marc Schmidt, Djemel Hamdane, Michael C. Marden, Thomas Hankeln and Thorsten Burmester

J. Biol. Chem. 2004, 279:24116-24122.
doi: 10.1074/jbc.M402011200 originally published online March 31, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M402011200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2004/04/07/M402011200.DC1

This article cites 47 references, 20 of which can be accessed free at
http://www.jbc.org/content/279/23/24116.full.html#ref-list-1