Gene 541 (2014) 60–66

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Transcriptional divergence of the duplicated hypoxia-inducible factor alpha genes in zebrafish

Kalle T. Rytkönen [*,1], Jenni M. Prokkola, Virpi Salonen, Mikko Nikinmaa

Division of Genetics and Physiology, Department of Biology, University of Turku, FI-20014 Turku, Finland

ARTICLE INFO

Article history:
Accepted 6 March 2014
Available online 7 March 2014

Keywords:
Oxygen
Gene duplication
Teleost
Tissue-specific transcription
Developmental transcription

ABSTRACT

Oxygen availability has been a major force in shaping the physiological evolution of animals. Under reduced oxygen availability (hypoxia) major changes in gene expression are mediated by hypoxia-inducible factors (HIF alphas). Tetrapods have three *hif alpha* genes, whereas zebrafish (*Danio rerio*) and other cyprinids have six due to a teleost lineage-specific genome duplication. We studied the transcriptional divergence of the six teleost-specific *hif alphas* by inspecting the tissue-specific transcription patterns in adult zebrafish and by monitoring the early developmental transcription of normoxia- and hypoxia-grown zebrafish embryos. Overall we observed the highest *hif alpha* mRNA levels in tissues that are important for hypoxic survival, including the brain, gill and heart. Of the paralogs that have not previously received attention (*hif alpha-1A*, *hif alpha-2B* and *hif alpha-3B*) especially the *hif alpha-2B* transcription levels suggest functional relevance. The *hif alpha-1A/B* paralogs that have considerable coding sequence divergence displayed more overall transcriptional divergence than the *hif alpha-2A/B* paralog pair. The *hif alpha-2A/B* paralogs that are similarly conserved in coding sequence had a divergent transcription pattern during early development. When zebrafish grown in modest hypoxia were compared to normoxia grown fish, only *hif alpha-3A* transcription was significantly altered. These results suggest that, in zebrafish, the evolutionary retention of each *hif alpha* paralog pair has involved unique patterns of coding sequence divergence, adult tissue-specific transcriptional divergence or developmental transcriptional divergence.
© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Oxygen has played a pivotal role in the adaptation of animals to terrestrial and aquatic environments. Reduced oxygen supply (hypoxia) causes major changes in gene expression, which are usually mediated by hypoxia-inducible factors (HIF alphas) (Kaelin and Ratcliffe, 2008; Lendahl et al., 2009; Semenza, 2012). The HIFs regulate the transcription of genes involved in a wide range of physiological processes, including glycolysis, glucose and iron transport, angiogenesis, erythropoiesis, and cell-cycle control (Lendahl et al., 2009; Wenger et al., 2005). In addition to coordinating the hypoxic response, HIFs also play important roles in embryonic development and organogenesis (Dunwoodie, 2009) and may be important in temperature responses of poikilothermic animals (Rissanen et al., 2006).

All vertebrates studied to date have at least three *HIF alpha* genes (Loenarz et al., 2011; Rytkönen et al., 2011). While HIF-1 alpha and HIF-2 alpha are the main transcription-activating HIF alphas, HIF-3 alpha is at least partly inhibitory to the actions of HIF-1 and HIF-2 (Kaelin and Ratcliffe, 2008; Lendahl et al., 2009). The main regulatory mechanism of the HIF system is post-translational oxygen-dependent modification of specific proline residues in HIF alphas (Kaelin and Ratcliffe, 2008; Lendahl et al., 2009; Semenza, 2012). Tissue specific transcription is responsible for constitutive *hif alpha* levels and studies in fishes (Chen et al., 2012; Law et al., 2006; Rytkönen et al., 2013) and hypoxia-tolerant mammals (Shams et al., 2004) suggest that HIF system is also transcriptionally regulated by hypoxia.

In contrast to the three *hif alphas* present in mammals, the *hif alpha* gene repertoire has been duplicated in a teleost lineage-specific genome duplication and consequently six full-length *hif alpha* gene copies (referred to as *hif-1A/B*, *hif-2A/B* and *hif-3A/B*) appear to have been retained only in cyprinid fishes (Rytkönen et al., 2013). Cyprinids (e.g. zebrafish (*Danio rerio*), minnows and carps) include several species that are more hypoxia- or even anoxia-tolerant than other vertebrates (Nilsson and Randall, 2010; Shoubridge and Hochachka, 1980). The duplicated *hif alpha* paralogs are retained in cyprinids partly because of adaptive subfunctionalization in their transcription patterns, e.g. while the transcription of *hif-1A* and *hif-2A* appears to be primarily developmentally regulated, *hif-1B* and *hif-2B* are regulated by hypoxia in adult zebrafish (Rytkönen et al., 2013).

While it is generally accepted that not only the early developmental transcriptional divergence, but also tissue-specific transcriptional

Abbreviations: Ct, critical threshold qPCR value; EP, Early Pharyngula; ES, Early Segmentation; HIF, hypoxia-inducible factor alpha; LG, Late Gastrula; LP–EH, Late Pharyngula–Early Hatching; qPCR, quantitative polymerase chain reaction.

* Corresponding author.
E-mail address: kalle.rytkonen@yale.edu (K.T. Rytkönen).
[1] Present address: Yale Systems Biology Institute, Department of Ecology and Evolutionary Biology, Yale University, 300 Heffernan Drive, West Haven, CT, 06516, USA.

http://dx.doi.org/10.1016/j.gene.2014.03.007
0378-1119/© 2014 Elsevier B.V. All rights reserved.

divergence is important for retaining duplicated genes (Conant and Wolfe, 2008; He and Zhang, 2005), these aspects of teleost specific *hif alphas* have not been fully assessed. Here, we study the transcriptional divergence of *hif alphas* by inspecting the tissue-specific transcription patterns in adult zebrafish and the early developmental transcription of both normoxia- and hypoxia-grown zebrafish embryos. We compare the divergence in the protein-coding regions of the *hif alpha* paralogs to the overall tissue-specific transcript levels and the developmental transcription profiles. Additionally, we inspect if there are sex-specific transcriptional differences in the studied tissues.

2. Materials and methods

2.1. Animal experiments and sampling

2.1.1. Tissue-specific sampling

Zebrafish were maintained at normoxia at 28 °C under a 14:10-h light–dark cycle and fed twice daily. Tissues were collected from three independent groups of adult males and three independent groups of adult females after fish were anesthetized with 0.1% 2-phenoxyethanol. Samples were collected from the brain, eye, gill, gonads, gut, heart, kidney, liver and muscle. RNA isolation and cDNA synthesis were performed as previously described (Kamei et al., 2008).

2.1.2. Developmental sampling

To examine the developmental transcription profiles, zebrafish embryos were grown on 35 mm petri dishes, with 25–30 embryos in 5 ml of E3 per dish in 3 independent biological replicates. Fertilized eggs were placed under either normoxia (ambient air) or in 50% air saturation from 1 to 2 hour post fertilization (hpf). Both normoxia and hypoxia embryos were collected during five specific developmental stages: Late Gastrula (LG), Bud, Early Segmentation (ES, 3–5 somites), Early Pharyngula (EP) and Late Pharyngula–Early Hatching (LP–EH). For the normoxia group these corresponded to 8, 10, 12, 24 and 48 hpf. Total RNA was extracted using TRI Reagent (Molecular Research Center, Inc., USA) according to the manufacturer's protocol. After DNase treatment (Macherey-Nagel, Germany, Cat. No. 740963) samples were cleaned and concentrated with NucleoSpin® RNA Clean-up XS kit (Macherey-Nagel, Germany) and RNA was quantified. 500 ng of total RNA was reverse transcribed to cDNA using DyNAmo™ cDNA Synthesis Kit (Finnzymes, Espoo, Finland) according to the manufacturer's protocol using oligo(dT) primers.

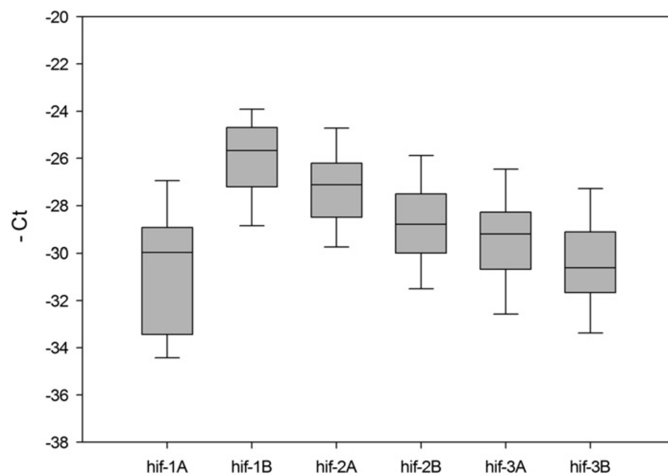

Fig. 1. The transcription levels of the six *hif alpha* gene paralogs across all the studied tissues. The inverse of qPCR raw threshold value (−Ct) reflects mRNA quantity in the sample. Whiskers note the data range across all tissues and the line marks the median.

2.2. Sequences, quantitative PCR and data analysis

The sequences and primers used in the study are displayed in Supplementary Table 1. Quantitative PCR (qPCR) primers were designed to span the longest introns using Roche server (www.roche-applied-science.com) and amplicons were verified by agarose gels and by sequencing. Reactions were run in triplicates on a 7900 HT Fast Real-Time PCR machine (Applied Biosystems). For the tissue samples qPCR 2× qPCR KAPA Master Mix with ROX was used with primers [500 nM], Universal Probe Library Taqman probes [1 μM]. cDNA was diluted 20× for the and 3 μl used per well. For the developmental series samples Fermentas Maxima SYBR Green qPCR Master Mix was used with standard cycling protocol and primers [100 nM]. Results were analyzed with SDS 2.4 (Applied Biosystems) using the standard curve method to transform raw Ct values to relative values. Standard curves were prepared as six 1:3 serial dilutions from a pooled cDNA sample. For Fig. 1 the transcription levels are shown as a box plot of raw Ct values in the tissue sample qPCR reactions, when the threshold was set to 0.05 for all of the genes, and for Figs. 2 and 3 as relative values. For tissue series the relative values were normalized to those of a reference gene *60S ribosomal protein L6* (*rpl6*) that we had previously tested to be relatively equally expressed across tissues (SD 1.7 cycles). For the hypoxia series, as commonly used reference genes are unreliable in normalizing gene transcription under hypoxia (Rytkönen et al., 2013), we used the amount of total RNA to normalize the transcript levels. The results were examined by t-tests or analysis of variance with pair wise testing. Statistical analysis and graphics were done using SigmaPlot11/12.

3. Results and discussion

3.1. Comparison of the protein coding divergence of Hif alpha paralogs with the overall mean transcription levels

To determine the protein coding sequence divergence of the teleost specific Hif alpha paralogs, we calculated the amino-acid identities using two out-groups that have not experienced the teleost-specific whole genome duplication (Table 1). The degrees of protein sequence divergence in zebrafish Hif-1A/B, Hif-2A/B and Hif-3A/B paralog pairs are different (Table 1). Zebrafish Hif-1A has diverged considerably more from the out-groups than Hif-1B (e.g. predicted amino acid identity compared to shark: Hif-1A 44.7% vs. Hif-1B 57.2%). In comparison, the two Hif-2 paralogs have approximately the same degree of divergence (compared to shark: Hif-2A 53.6% and Hif-2B 56.1% identity). Among the six hif alpha paralogs, Hif-3B shows the highest levels of divergence. It has only 23.6–27.1% identities to the shark and tetrapod Hif-3.

Next, we examined the overall transcription patterns of all six teleost-specific *hif alpha* paralogs across zebrafish tissues. All *hif alpha* paralogs were transcribed in the zebrafish tissues examined. Fig. 1 shows the mean levels and the variation of the *hif alpha* transcripts across all the studied tissues. *Hif-1B* is abundantly transcribed in all tissues, whereas *hif-2A*, *hif-2B* and *hif-3A* have intermediate levels of transcription. *Hif-3B* has low but clearly detectable transcription levels, while *Hif-1A* only just reaches the detection limit in certain tissues. The biggest difference in mRNA levels within a given paralog pair is seen between *hif-1A* and *hif-1B*: *hif-1B* mRNA is on average ca. 20 fold more abundant than that of *hif-1A*. In comparison, the *hif-2* and *hif-3* paralog pairs have more similar mean transcription levels. Within each paralog pair, we compared these divergences in the mean mRNA levels with the protein-coding region divergences from corresponding tetrapod or shark copies (Table 1). The *hif-1A/B* paralog pair that has considerable coding sequence divergence displayed more transcriptional divergence than the *hif-2A/B* paralog pair whose coding sequences have the same degree of relatively high conservation. However, for

Download English Version:

https://daneshyari.com/en/article/2816393

Download Persian Version:

https://daneshyari.com/article/2816393

Daneshyari.com