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The 14-3-3 proteins in the teleost fish rainbow trout (*Oncorhynchus mykiss*)

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Summary

We report studies of *14-3-3* genes in rainbow trout, adding to current understanding of the molecular evolution of this multigene family and its functional importance in fish. Ten genes were identified that are apparent duplicates of five ancestors. The duplicated *14-3-3* genes diverged rapidly and their cladogram is markedly different from the phylogenetic tree. The mean rate of nonsynonymous divergence of trout *14-3-3* genes is one order of magnitude greater than that of mammalian genes. An evolutionarily recent genome duplication in salmonid fish relaxed functional constraints, and selection favored establishment of novel isoforms. Differences in tissue distribution of *14-3-3* genes were minor; however, results of 31 microarray experiments showed divergence of expression profiles, which was related to structural divergence of the duplicates. We observed remarkable coordination of expression of all isoforms in our study of stress response in the brain. Profiles of the *14-3-3* genes correlated with a large group of chaperones and genes involved in cell communication and signal transduction. We studied embryonic expression of *14-3-3* genes and found abundant transcripts in the rapidly growing and differentiating parts of embryos such as eyes, tail bud and yolk syncytium during somitogenesis and in gills and pectoral fins after completion of somitogenesis. Consistent expression was observed in the neural crest, which is known to have high morphogenetic potential.

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Key words: 14-3-3 proteins, rainbow trout, *Oncorhynchus mykiss*, duplicated genes, phylogeny, expression.

Introduction

The 14-3-3s are small acidic proteins that are found in all eukaryotic organisms (reviewed in Aitken et al., 1992; Burbelo and Hall, 1995; Finnie et al., 1999; Baldin, 2000; Tzivion and Avruch, 2002; van Hemert et al., 2001; Ferl et al., 2002; Masters et al., 2002). The homodimers or heterodimers of 14-3-3 proteins interact with client proteins through the amphipathic groove in the middle region, which alters conformation of the bound ligand. This may affect activity of regulated proteins or their ability to interact with other protein partners, modify their intracellular distribution and/or protect from proteolysis and dephosphorylation (reviewed in Tzivion and Avruch, 2002). The number of known partners of 14-3-3 is already greater than 100 (van Hemert et al., 2001). Analyses of plant protein sequences for the conserved binding domains suggested that as much as 20% of *Arabidopsis* proteins are potential clients of 14-3-3s (Sehnke et al., 2002). Interaction with a wide range of partners accounts for the diversified functions of the 14-3-3 proteins. They are involved in signal transduction, checkpoint and cell cycle control, apoptosis, gene transcription, chromosome remodeling, regulation of metabolism, intracellular protein trafficking and stress response.

Taking into account remarkable structural conservation of the *14-3-3* genes (human and yeast proteins share 70% similarity), the existence of a large number of isoforms may seem surprising. Thirteen *14-3-3* genes were found in the genome of *Arabidopsis*, and expression of 12 isoforms has been confirmed (Ferl et al., 2002). In mammals, there are seven distinct 14-3-3 proteins. One gene belongs to the epsilon type, which is common to plants and animals; the others are denoted as sigma, gamma, eta, beta, zeta and tau. Are the functions of the *14-3-3* gene products redundant or does each gene have a unique function? Microarray analyses and sequencing of cDNA libraries showed that most human tissues harbor several or all of the 14-3-3 proteins, which do not have any marked tissue specificity [expression profiles are deposited in the GeneCards database (http://bioinfo.weizmann.ac.il/cards/)]. In most *in vitro* studies, 14-3-3 proteins bound ligands with similar affinities. Although several client proteins showed

preferential interaction with some of the 14-3-3 isoforms, the biological relevance of subtle differences in affinities remains unknown (reviewed in Fu et al., 2000). At present, there is not much evidence for the functional divergence of animal 14-3-3 isoforms. Studies of lower vertebrate 14-3-3 proteins are important for understanding of evolution and structural and functional diversification of this gene family. To date only three non-mammalian vertebrate *14-3-3* genes have been characterized. Two genes were identified in *Xenopus laevis* tadpoles (Kousteni et al., 1997; Kumagai et al., 1998) and one *14-3-3* gene was characterized in the teleost fish *Fundulus heteroclitus* (Kultz et al., 2001). In the present study, we present 10 *14-3-3* genes from rainbow trout, which are likely to be duplicates of five ancestral genes. Their structure, evolution and expression are reported.

Materials and methods

Libraries and clones

A subtracted cDNA library was prepared from the brain of stressed rainbow trout. Fingerlings (40–50 g) were stressed by netting (2 min) followed by confinement (20 min). This treatment was repeated over five consecutive days. RNA was extracted with Trizol reagent (Invitrogen, Paisley, UK), and mRNA was purified with Dynabeads kit (Dynal, Oslo, Norway). Direct and reverse suppressive subtracted hybridization was performed as described in Diatchenko et al. (1999). PCR-amplified cDNA was cloned into pcdna3.1 vector (Invitrogen), and a *14-3-3* gene was identified among the cDNA sequences. Additional expressed sequence tags (ESTs) having homology to 14-3-3 proteins were identified from a large number of ESTs derived from a single normalized cDNA library constructed from rainbow trout brain, gill, kidney, spleen, liver and muscle mRNA (Rexroad et al., 2003). These clones included complete reading frames.

Sequence analyses

EST sequences were analyzed with stand-alone blast (Altschul et al., 1997). Multiple sequence alignments were performed with ClustalW (Thompson et al., 1994). Synonymous and nonsynonymous substitutions in genes were determined with Dnasp (Rozas and Rozas, 1999). Phylogenetic analyses were performed with Phylip (Felsenstein, 1989). After alignment of protein sequences, 1000 bootstrap datasets were generated and Dayhoff's PAM matrix was computed. The consensus trees were constructed with the neighbor-joining method. The rainbow trout, *Oncorhynchus mykiss* (*Omy*), and zebrafish, *Danio rerio* (*Dr*), 14-3-3 proteins were denoted with respect to the result of phylogenetic study.

Expression in embryos and tissues of juvenile rainbow trout

Expression patterns of *Omy14-3-3* genes in embryos and tissues of rainbow trout were analyzed with RT-PCR. The PCR primers (Table S1 in supplementary material) were designed from cDNA sequences to specifically amplify diverged fragments.

In situ *hybridization*

Anti-sense probes were prepared for the 3′-UTRs of *Omy14-3-3* genes. Plasmids were PCR amplified with gene-specific primers aligning to the ends of protein coding sequences (Table S1 in supplementary material) and an universal M13 primer. *In vitro* transcription was conducted using T7 RNA polymerase (MBI Fermentas, Vilinius, Lithuania) and DIG RNA labeling mix (Roche Bioscience, Indianapolis, IN, USA). Samples were collected at the 40-somite stage and immediately after completion of somitogenesis. Embryos were processed as described in Joly et al. (1993). The probes were detected with AP-conjugated antibodies using NBT/BCIP (nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate) substrate (Roche Bioscience). Pictures were taken with an Olympus CK40 microscope.

Microarrays

The *Omy14-3-3* genes were selected for inclusion onto a glass microarray containing 1300 rainbow trout and Baltic salmon (*Salmo salar*) genes, and each clone was spotted in six replicates. Multiple gene expression profiling was performed in experiments that addressed genomic response to stress, toxicity, bacterial antigens and adaptation to cold. RNA was extracted with Trizol reagent (Invitrogen), and four fish were pooled in each sample. Labeling with Cy3- and Cy5-dCTP (Amersham Pharmacia, Little Chalfont, UK) was made using SuperScript III reverse transcriptase (Invitrogen) and oligo(dT) primer; cDNA was purified with Microcon YM30 (Millipore, Bedford, MA, USA). We used a dye-swap experimental design (Kerr and Churchill, 2001). Each sample was hybridized to two microarrays. For the first slide, test and control cDNA were labeled with, respectively, Cy5 and Cy3. For the second array, dye assignment was reversed. Slides were prehybridized with 1% bovine serum albumin (BSA), fraction V, 5×SSC and 0.1% sodium dodecyl sulfate (SDS) (30 min at 50°C) and washed with 2×SSC (3 min) and 0.2×SSC (3 min). Arrays were hybridized overnight; hybridization cocktail contained 1.3×Denhardt's, 3×SSC, 0.3% SDS, 0.67 μg μl^{-1} polyadenylate and 1.4 μg μl^{-1} yeast tRNA (Sigma-Aldrich, St Louis, MO, USA). Slides were washed with 0.5×SSC, 0.1% SDS (15 min), 0.5×SSC, 0.01% SDS (15 min) and twice with 0.06×SSC (2 min). Scanning was conducted with ScanArray 5000 (Perkin Elmer–Wallac, Turku, Finland), and images were processed with QuantArray (GSI Luminomics, Munich, Germany). The spot measurements were filtered by criteria $I–B>3$ and $(I–B)/(S_I+S_B)>0.6$, where I and B are the mean signal and background intensities, respectively, and S_I and S_B are their standard deviations. After subtraction of mean background, lowess normalization was performed. Divergence of expression profiles of *14-3-3* genes was analyzed using results of 31 microarray experiments. For every pair, we estimated the number of genes that significantly ($P<0.05$) correlated with both (Pearson r). Then, similarity matrices were analyzed with multidimensional scaling. Genes that significantly correlated with all *14-3-3* isoforms were determined to

characterize the stress response of the brain. Enrichment of the functional classifications of these transcripts was observed by analysis with Ease (Hosack et al., 2003); significance was determined with exact Fisher's test (P<0.05).

Results

Identification and structural characterization of rainbow trout 14-3-3 *genes*

The first *Omy14-3-3* gene was found in the subtracted library prepared from stressed rainbow trout brains and was observed to be similar to the mammalian beta protein. To identify other isoforms, we compared the sequences of 47395 rainbow trout ESTs from the normalized rainbow trout library with human 14-3-3 proteins using stand-alone blastx (Altschul et al., 1997). Blastn distributed the clones encoding putative *14-3-3* genes into 10 clusters, which contained identical sequences of different length. Furthermore, we sequenced 10 clones whose 5′-ends included predicted start codons. We analyzed all salmonid fish ESTs deposited in National Center for Biotechnology Information (NCBI) dbEST (http://www.ncbi.nlm.nih.gov/dbEST/) and The Institute for Genomic Research (TIGR; http://www.tigr.org/tdb/tgi/rtgi/) and did not find any new isoforms. Multiple sequence alignment with ClustalW (Thompson et al., 1994; http://www.ebi.ac.uk/clustalw/) divided 10 *Omy14-3-3* genes into five pairs, which were designated as *A*, *B* (similar to beta), *C*, *E* (similar to epsilon) and *G* (similar to gamma). The protein coding sequences were highly similar in every pair (Fig. 1), whereas the 5′- and 3′-UTRs were divergent in all genes except *Omy14-3-3A*.

For phylogenetic analyses, we used 14-3-3 proteins of teleost fish (*Fundulus* and *Danio*) and mammals whose sequences were retrieved from Swiss-Prot (http://us.expasy.org/sprot/); the outroot was from ascidia (*Ciona intestinalis*). In addition to five Dr14-3-3 proteins from Swiss-Prot, five sequences were available from Ensembl (http://www.ensembl.org/) and four more were found by comparison of UniGene EST clusters (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene) with human 14-3-3 proteins using blastx (Table S2 in supplementary material). The total number of putative *Dr14-3-3* genes was 14; however, two short sequences were not included in the analyses. Phylogenetic study provided additional evidence for duplication of ancestral rainbow trout genes, since 10 Omy14-3-3 proteins were divided into five separate clades (Fig. 2). Ten of 12 *Dr14-3-3* genes were duplicates, and three *Dr14-3-3G* genes could arise as a result of two subsequent duplications. Some cyprinids are, like salmonids, tetraploid, and the possibility of ancient genome duplication in zebrafish is debated. All types of Omy14-3-3 proteins were found in zebrafish. In rainbow trout, there was no ortholog to the *Fundulus* (F) gene; however, this was assigned to the clade including Dr14-3-3F1 and F2. Three of six types of fish 14-3-3 proteins (E, G and B) were clustered with mammalian epsilon, gamma and beta proteins at high bootstrap values. The A, C and F types were specific for fish, whereas zeta, sigma, eta and tau 14-3-3 proteins were found exclusively in mammals. Interestingly, in four of five pairs of Omy14-3-3 proteins (B, C, G and E), one gene was assigned to a clade containing the second gene and either the mammalian or zebrafish ortholog. The 14-3-3 cladogram was markedly different from the organismic tree and this suggested rapid divergence of duplicated *Omy14-3-3* genes. To verify this finding, we compared synonymous (K_s) and nonsynonymous (K_a) divergence in rainbow trout and

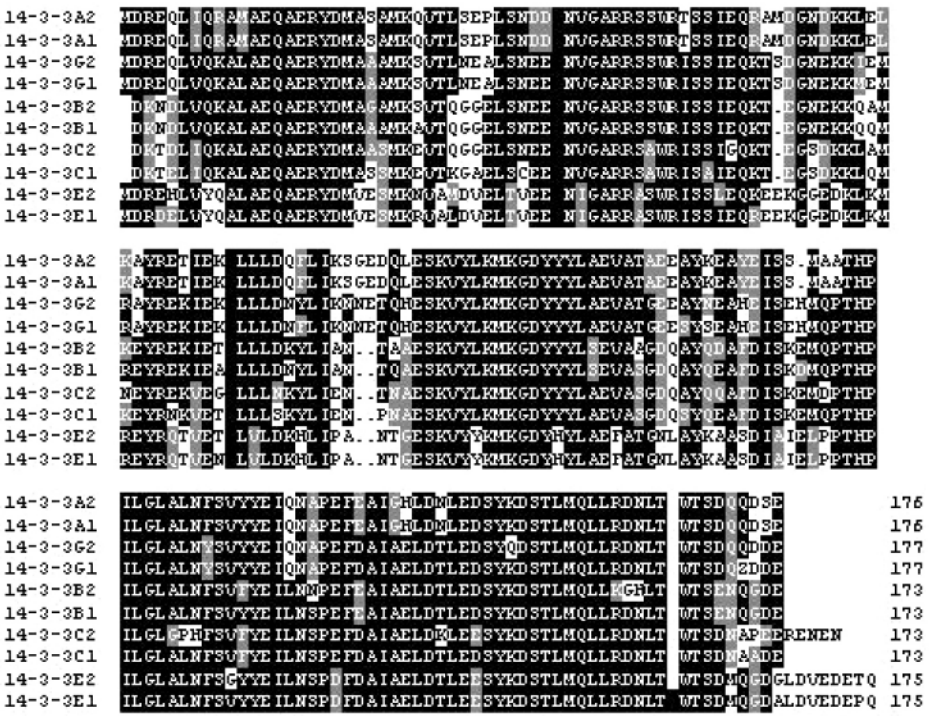

Fig. 1. Structure of rainbow trout 14-3-3 proteins. The sequences were aligned with ClustalW and the conserved amino acids were highlighted with Boxshade.

mammalian (human and mouse) *14-3-3* genes (Table 1). The mean K_s in fish genes was 1.65 times greater than in mammalian *14-3-3* genes, whereas K_a was increased 10.5-fold.

Expression of rainbow trout 14-3-3 *genes*

Expression of *14-3-3* genes in tissues of rainbow trout was analyzed by RT-PCR. The PCR primers were designed to distinguish between the duplicated genes; however, we were unable to separate the *Omy14-3-3A1* and *A2* isoforms due to their high sequence similarity. Distribution of *14-3-3* genes was ubiquitous, and transcripts of six genes (*Omy14-3-3B1*, *B2*, *C1*, *C2*, *G1* and *G2*) were found in 10 of 11 analyzed tissues (Fig. 3). Brain, ovary and testis harbored a complete set of *Omy14-3-3* genes. The number of expressed isoforms in other tissues ranked from eight (gill and kidney) to two (skin). Expression of *Omy14-3-3* genes was also studied in embryos. *Omy14-3-3B1* and *B2* were expressed at all analyzed developmental stages (Fig. 4). Three more genes (*Omy14-3-3C1*, *C2* and *A*) were detectable in blastulas. This could be due to persistence of maternal transcripts, because expression of these genes was interrupted at the subsequent developmental stages. Six of nine analyzed *Omy14-3-3* genes were already expressed in the late gastrulas. Expression of *Omy14-3-3E2* and *C2* began at early somitogenesis (15 somites), whereas *G2* was the latest isoform (34 somites).

We analysed differential expression of *Omy14-3-3* genes in 31 microarray experiments that dealt with response of rainbow trout to stress, environmental pollutants and bacterial antigens. Isoforms were compared by correlation of expression profiles with other genes presented on the slide and the data were analysed with multi-dimensional scaling. Distance metrics were calculated for every pair of duplicated genes and differences of expression profiles were clearly related to the non-synonymous divergence of duplicates (Fig. 5; Table 1). However, it is possible that difference of expression between the *A1* and *A2* isoforms was underestimated. In contrast to other *Omy14-3-3* genes, the 3′-UTR sequences of these mRNAs are highly conserved, and cross-hybridization could affect the results of microarray analyses. We observed tight coordination of expression of 10 isoforms in studies of stress response in the rainbow trout brain. All *Omy14-3-3* genes were downregulated after 1 day, which was followed by a subsequent increase of expression levels (Fig. 6). We selected genes with significantly similar profiles (Pearson r, $P<0.05$) and analyzed over-representation of Gene Ontology functional classes (Ashburner et al., 2000) in this group (Table 2). This result suggested that *Omy14-3-3* genes could share regulatory mechanisms with nuclear proteins, chaperones and proteins involved in signal transduction, nucleotide binding and metabolism.

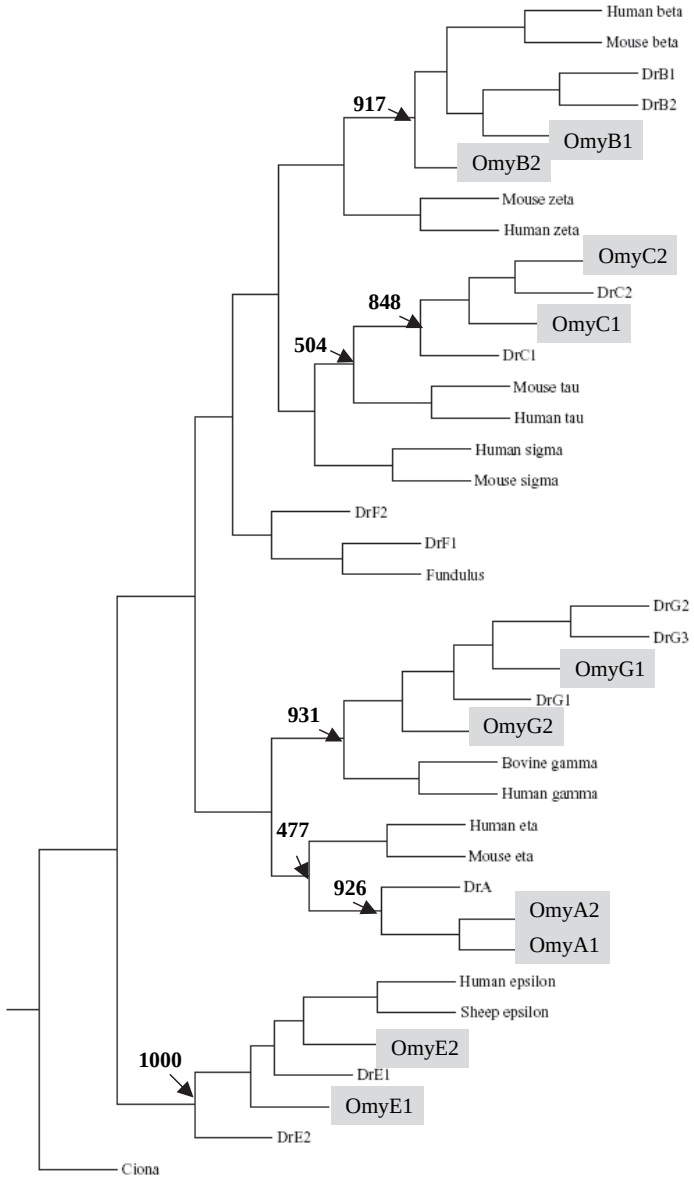

Fig. 2. Cladogram of vertebrate 14-3-3 proteins. Protein distant matrix was computed and the tree was constructed using the neighbor-joining method. The bootstrap values for 1000 replicates are indicated at the nodes of clades that include rainbow trout proteins. Accession numbers of proteins used in phylogenetic analyses are given in Tables S2, S3 (supplementary material).

Table 1. *Synonymous (K_s) and nonsynonymous (K_a) divergence in* 14-3-3 *genes*

Genes	K_s	K_a
Rainbow trout		
14-3-3A	0.182	0.0074
14-3-3G	0.624	0.0243
14-3-3E	1.023	0.0266
14-3-3B	0.689	0.0539
14-3-3C	1.038	0.0866
Mammals		
14-3-3 gamma	0.437	0
14-3-3 epsilon	0.127	0
14-3-3 eta	0.704	0.0035
14-3-3 tau	0.322	0.0035
14-3-3 zeta	0.391	0.007
14-3-3 beta	0.597	0.0087

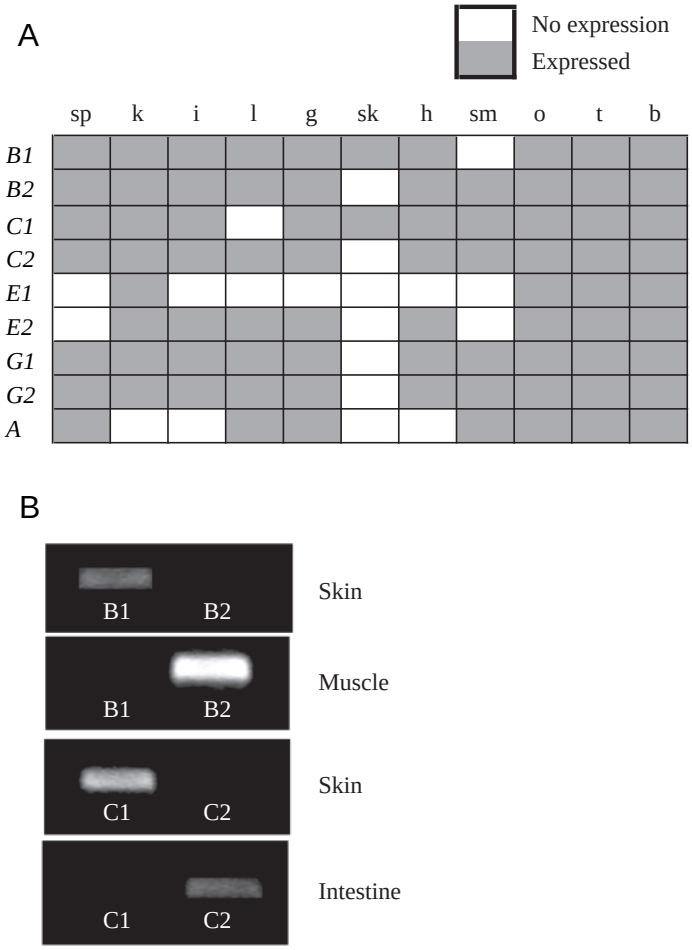

Fig. 3. (A) Expression of *Omy14-3-3* genes was analyzed with RT-PCR in rainbow trout spleen (sp), kidney (k), intestine (i), liver (l), gill (g), skin (sk), heart (h), skeletal muscle (sm), ovary (o), testis (t) and brain (b). In each sample, RNA was pooled from three individuals and analyses were repeated twice. (B) The gel picture presents differential expression of duplicated *Omy14-3-3* genes.

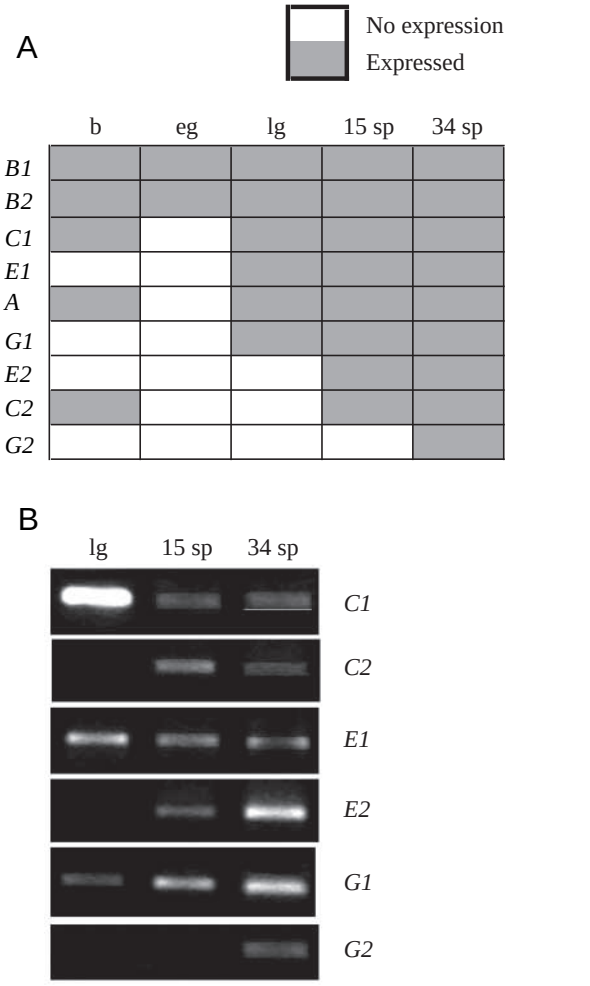

Fig. 4. (A) Expression of *Omy14-3-3* genes in rainbow trout embryos was determined with RT-PCR at different developmental stages: blastula (b), early gastrula (eg), late gastrula (eg), 15 somites (15 sp) and 34 somites (34 sp). In each sample, RNA was pooled from 10 embryos and analyses were repeated twice. (B) The gel picture presents differential expression of duplicated *Omy14-3-3* genes.

Expression of six *Omy14-3-3* genes (*A1*, *A2*, *E1*, *E2*, *G1* and *G2*) in somitic (40 somites) and postsomitic embryos was analyzed with *in situ* hybridization. To separate closely related isoforms, we used the PCR-amplified 3′-UTRs as templates for preparation of probes. We were unable to find any marked difference in the expression patterns of *Omy14-3-3* genes; therefore *Omy14-3-3B1* is shown as an example (Fig. 7). In somitic embryos (Fig. 7A,B), transcripts were found in the neural crest, eyes, yolk syncytium, tail bud and caudal somites. Interestingly, expression of *14-3-3* genes in the tail bud and caudal somites was seen in some of the analyzed embryos. In postsomitic embryos (Fig. 7C,D), transcripts were detected in the neural crest, gill covers and gill arches and in pectoral fins; however, there was no expression in the tail and eyes.

Discussion

The number of *14-3-3* isoforms in complex organisms, such as mammals and seed plants, may seem striking taking into account the exclusively high degree of sequence conservation of these proteins. Identification of 10 *14-3-3* genes in bony fish rainbow trout provided additional evidence for diversity of this multi-gene family in vertebrates. Bony fish and mammals have different sets of 14-3-3 proteins. We were unable to find orthologs to four of seven types of mammalian 14-3-3 proteins among more than 527 000 zebrafish and salmonid ESTs. In this study, we identified five types of rainbow trout 14-3-3 proteins and one more gene that was previously characterized in the teleost fish *Fundulus* (Kultz et al., 2001). Of six fish protein types, one (epsilon) is common to plants and animals and two more (beta and gamma) are shared by fish and mammals. Probably, these were established before segregation of teleosts and tetrapods. Fish C and A proteins were clustered with mammalian tau and eta; however, low bootstrap values did not allow us to regard these genes as orthologs (Fig. 2). Finally, type F protein was clearly distinct from any of the mammalian

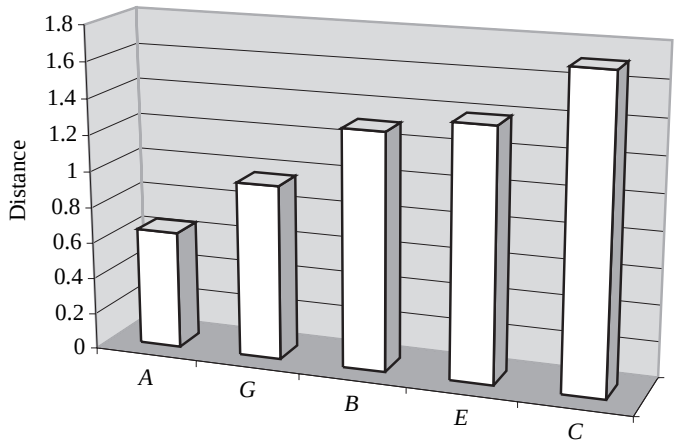

Fig. 5. Duplicated *Omy14-3-3* genes were compared by similarity of expression profiles in 31 microarray experiments. The distances were determined with multidimensional scaling.

Table 2. *Significantly over-represented functional classes (exact Fisher's test,* P*<0.05) in the list of genes whose expression correlated with* Omy14-3-3 *genes in the brain of stressed rainbow trout*

Functional classes	Number of genes
Nucleus	47
Cell communication	39
Signal transduction	33
Nucleotide binding	28
Intracellular signaling cascade	17
Chaperone activity	12
Small GTPase mediated signal transduction	7
Receptor signaling protein activity	6
Purine ribonucleotide biosynthesis	4
Chromatin binding	4

genes. One may conclude that the appearance of novel 14-3-3 proteins was parallel to the origin of vertebrate classes. All mammalian 14-3-3 proteins were found in different species, whereas six types of fish 14-3-3 proteins were present in at least two phylogenetically remote groups.

Continuous emergence of novel 14-3-3 isoforms in the vertebrate evolution suggested a strong tendency towards functional diversification of this multi-gene family. This was supported by rapid divergence of rainbow trout genes. All *Omy14-3-3* genes seem to be duplicates, and at least four of five pairs most likely appeared due to the duplication of the salmonid genome, which took place about 50 million years ago (Bailey et al., 1978). It is possible that duplication of *Omy14-3-3A* occurred independently and later, since sequence divergence in this pair was markedly lower than in other genes (Fig. 1; Table 1). In organisms of tetraploid origin, functionally redundant duplicated genes are gradually silenced, becoming pseudogenes in a process referred to as diploidisation. Active genes may evolve in a neutral mode or, alternatively, they are selected for conservation or divergence. In rainbow trout, all duplicated *14-3-3* genes are expressed at relatively high level. Phylogenetic analysis suggested that in four of five pairs of *Omy14-3-3* genes one was closer to the mammalian or zebrafish ortholog than to the homologous gene. The only exclusion was the least diverged *Omy14-3-3A* pair. A similar result was reported by Taylor et al. (2001) for nine of 27 analyzed duplicate zebrafish genes. Furthermore, nonsynonymous divergence in the duplicated rainbow trout *14-3-3* genes was one order of magnitude greater than in human and mouse *14-3-3* genes. Structural conservation of orthologous *14-3-3* genes in the phylogenetically remote mammalian species is likely to be due to their indispensable cellular functions. Duplication of genes can relax these constraints, since one isoform is sufficient for preservation of function whereas the second one can acquire novel features.

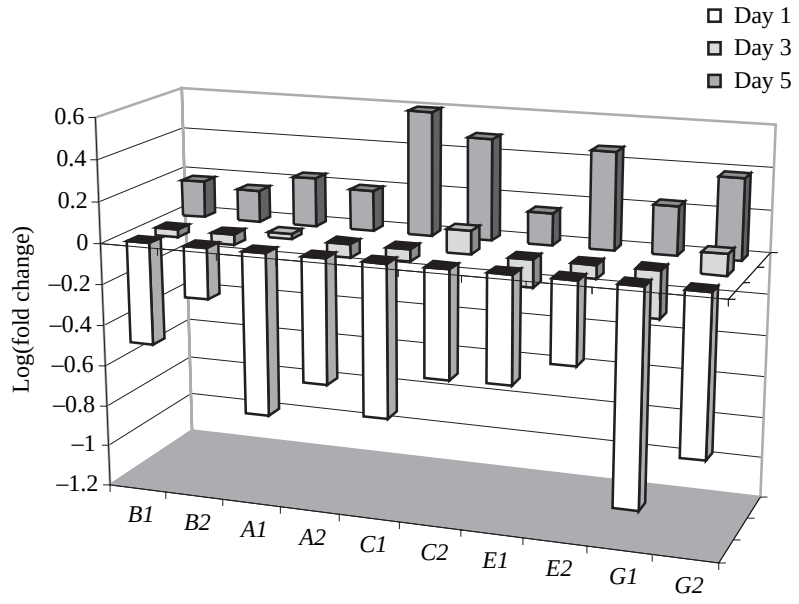

Fig. 6. Expression of *Omy14-3-3* genes in the brain of stressed rainbow trout. One-year-old fish were stressed by netting (2 min) and confinement (20 min) and this treatment was repeated over 5 days. The brain samples were collected 1, 3 and 5 days after the first stress exposure. In each sample, RNA was pooled from four individuals. A dye-swap design was used for hybridization, and expression of genes was measured in 12 replicates.

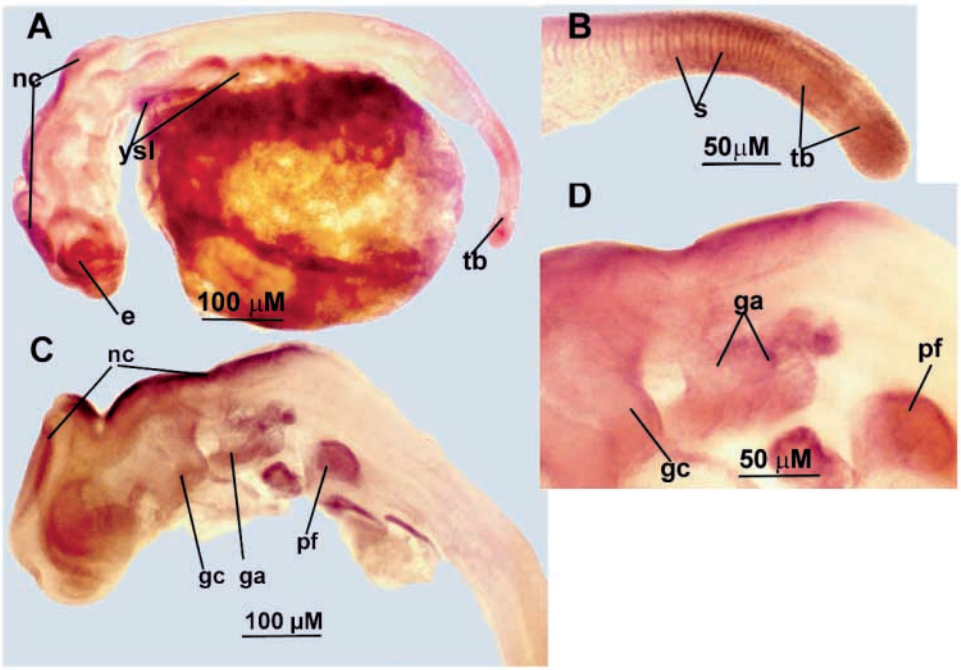

Fig. 7. Expression of *Omy14-3-3B1* was analyzed in somitic (A,B) and postsomitic (C,D) rainbow trout embryos with *in situ* hybridization. 15 embryos were analyzed at each developmental stage, and representative examples are shown. Transcripts were detected in the neural crest (nc), eyes (e), yolk syncytium layer (ysl), caudal somites (s), tail bud (tb), gill covers (gc), gill arches (ga) and pectoral fins (pf). The areas of active expression are shown at higher magnification in B and D.

Activity and rapid divergence of the duplicated rainbow trout *14-3-3* genes strongly suggests that appearance of new distinct isoforms was favored.

Rapid divergence of rainbow trout 14-3-3 proteins suggests functional diversification of the duplicated genes. Microarray analyses in yeast showed differential expression in most pairs with nonsynonymous divergence (Gu et al., 2002). Recent study of the zebrafish *annexin* gene family found that difference in expression patterns of duplicated genes was closely related to the degree of their sequence divergence (Farber et al., 2003). Analyses of tissue distribution and embryonic expression of *Omy14-3-3* revealed only minor differences between isoforms. Thus, expression of *Omy14-3-3C1* but not of *C2* was found in the intestine whereas the opposite was observed in skin (Fig. 3B). One of two *Omy14-3-3B* isoforms was active in skin (*B1*) and in muscle (*B2*). In embryonic development, *Omy14-3-3E1* and *G1* were activated earlier than *Omy14-3-3E2* and *G2*, respectively (Fig. 4). Furthermore, we compared expression profiles of *Omy14-3-3* genes in microarray experiments. Distance between isoforms was determined by numbers of genes that showed similar expression profiles with both genes of each pair and it decreased in the range *C>E=B>G>A*. Noteworthy, the duplicated genes were ranked in nearly the same order by non-synonymous divergence (Table 1). This result indicated that structural diversification of the duplicated genes could be due to adaptation to different client proteins.

In most microarray experiments, differential expression was shown by one or several *Omy14-3-3* isoforms. We demonstrated close concordance of expression profiles of all 10 *Omy14-3-3* genes in the brain of stressed rainbow trout (Fig. 6), and similar responses were shown by a large group of genes, which were significantly over-represented by nuclear proteins and proteins involved in cell communication and signal transduction. In line with this finding, we observed coordinated expression of all *Omy14-3-3* isoforms in parts of embryos that are known for rapid growth and differentiation. Stable expression was seen in the neural crest, which is characterized by exclusively high morphogenetic potential. This structure gives rise to connective tissue, some muscle, dermal and pigmented tissues and many structures including the cranium, branchial skeleton and sensory ganglia (reviewed in Baker and Bronner-Fraser, 1997; Gorodilov, 2000). We did not find tail bud expression after completion of somitogenesis. Noteworthy, only some of the analyzed somitic embryos expressed *Omy14-3-3* genes in the tail buds, which could indicate cyclic activity of these genes. At the subsequent stages, expression began to decrease in most tissues and the marked differences were observed between early and differentiated mesenchyme.

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