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Rapidly evolving fish genomes and teleost diversity

Vydianathan Ravi[a] and Byrappa Venkatesh[a,b]

Teleost fishes are the largest and most diverse group of vertebrates. The diversity of teleosts has been attributed to a whole-genome duplication (WGD) event in the ray-finned fish lineage. Recent comparative genomic studies have revealed that teleost genomes have experienced frequent gene-linkage disruptions compared to other vertebrates, and that protein-coding sequences in teleosts are evolving faster than in mammals, irrespective of their duplication status. A significant number of conserved noncoding elements (CNEs) shared between cartilaginous fishes and tetrapods have diverged beyond recognition in teleost fishes. The divergence of CNEs seems to have been initiated in basal ray-finned fishes before the WGD. The fast evolving singleton and duplicated genes as well as the divergent CNEs might have contributed to the diversity of teleost fishes.

Address
[a] Institute of Molecular and Cell Biology, A*STAR (Agency for Science, Technology and Research), Biopolis, Singapore 138673, Singapore
[b] Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119074, Singapore

Corresponding author: Venkatesh, Byrappa (mcbbv@imcb.a-star.edu.sg)

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Introduction

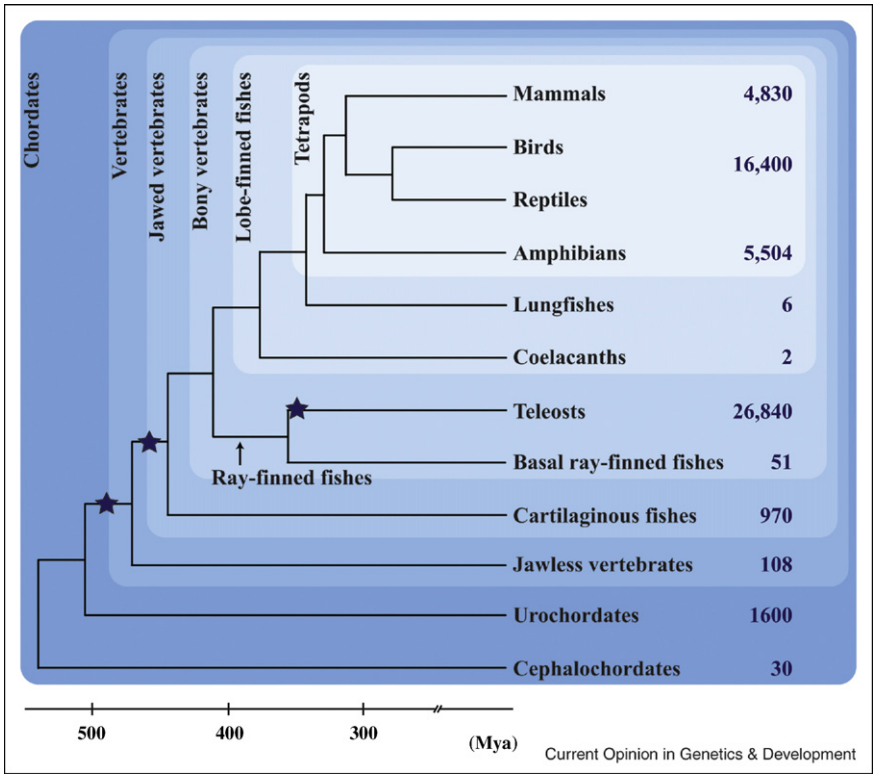
Teleost fishes, with about 27 000 species [1], are the largest and most diverse group of vertebrates. They exhibit remarkable diversity in their morphology, behavior, and adaptations. Teleosts account for more than 99% of ray-finned fishes (Actinopterygians) which diverged from lobe-finned fishes (Sarcopterygians) about 420 million years ago (Mya). The remaining ray-finned fishes, which are basal to teleosts, are represented by only ~50 living species (Figure 1). The sequencing of genes and gene families from teleost fishes in the pregenomic era had unexpectedly revealed the presence of duplicate teleost genes for several human genes. This led to the hypothesis that a whole-genome duplication (WGD) occurred in the ray-finned fish lineage before the diversification of teleost fishes [2–4]. More recently, the sequencing and comparative analysis of whole-genome sequences of teleost fishes such as fugu, *Tetraodon*, and medaka [5,6,7••] have provided compelling evidence for the WGD event in the fish lineage. Timing the gene duplication events using molecular clock and phylogenetic analyses indicated that the duplication occurred around 350 Mya, before the diversification of teleosts [8–11]. Since the basal ray-finned fishes are relatively species-poor, and the WGD predated the teleost radiation, the diversity and complexity of teleost fishes is often attributed to the WGD [10–14]. However, it has been pointed out that when the number and complexities of the extinct ray-finned fish taxa are also considered, there is no correlation between the fish-specific genome duplication and the diversity of teleost fishes [15]. Nonetheless, since major WGD events during vertebrate evolution are preceded by a series of extinction events, it is argued that the WGD could have reduced the probability of extinction through high evolvability, leading ultimately to the increased diversification of the surviving taxa [16]. Besides verifying the fish-specific WGD, recent studies have shown that teleost fishes have experienced a higher rate of chromosomal rearrangements and a faster evolution of protein sequences and conserved noncoding elements (CNEs) compared to cartilaginous fishes and mammals. Here, we review these data and discuss their implications for the evolution and diversity of teleost fishes.

Higher rate of chromosomal rearrangements in fishes

A WGD is generally followed by an increased rate of interchromosomal rearrangements [17]. A comparison of the recently completed medaka genome with the zebrafish, *Tetraodon*, and human genomes has revealed that eight major interchromosomal rearrangements occurred within a relatively short period of ~50 million years after the WGD in the fish lineage [7••]. Subsequently, while the medaka lineage experienced no major interchromosomal rearrangements, three major rearrangements occurred in the *Tetraodon* lineage. In contrast, the zebrafish lineage has experienced many interchromosomal rearrangements after it diverged from the medaka lineage [7••]. These results suggest that the WGD led to a transient increase in the rate of interchromosomal rearrangements and subsequently the teleost genomes are experiencing differential rates of chromosomal rearrangements.

An analysis of disruption of gene-linkages in vertebrates has suggested that teleost fishes have experienced a higher rate of chromosomal rearrangements compared to other vertebrates. Two studies that used different

Figure 1



Phylogenetic relationships of chordates. The number of extant species in the respective group (based on Ref. [1]) is given on the right. Stars represent the three major WGD events. Two of them occurred at the base of vertebrates, with the first occurring before the divergence of cyclostomes (jawless vertebrates) and the second occurring most likely after the divergence of cyclostomes [35]. The third WGD occurred in the teleost ancestor. Basal ray-finned fishes include the orders Polypteriformes (bichirs), Acipenseriformes (sturgeons and paddlefish), Semionotiformes (gar), and Amiiformes (bowfin). The scale bar represents fossil-based minimum divergence times: jawless vertebrates (Agnatha) and jawed vertebrates (Gnathostomes), 477 Mya [45]; cartilaginous fishes (Chondrichthyes) and bony vertebrates (Osteichthyes), 450 Mya [46]; ray-finned fishes (Actinopterygii) and lobe-finned fishes (Sarcopterygii), 416 Mya [47].

approaches to estimate the rate of gene-linkage disruption have found that the frequency of synteny loss in the terminal branches of teleosts is considerably higher than in the tetrapod lineages [18•,19••]. Recently, a 1.4× coverage sequence representing approximately 75% of the genome of a cartilaginous fish, the elephant shark (*Callorhinchus milii*), was generated based mainly on paired-end sequences of fosmid clones [20••]. A comparison of syntenic genes in the elephant shark (genes that map to the paired ends of a fosmid clone) with their orthologs in human and zebrafish genomes showed that the level of conserved synteny between the elephant shark and zebrafish is less than half of that between the elephant shark and human, and that the conserved clusters of syntenic genes in zebrafish contain fewer genes than in elephant shark and human [20••]. Although the loss of conserved synteny in teleosts can be because of the reciprocal loss of paralogous genes in duplicate chromosomes, comparisons of gene maps have indicated a high frequency of intrachromosomal rearrangements since the divergence of teleost and tetrapod ancestors [21,22]. Moreover, a comparison of gene order across large regions provides support for a higher rate of chromosomal rearrangements in teleost fishes compared to other vertebrates [23,24•]. For example, in contrast to the tight linkage between the human genes for the neurohypophysial hormones, oxytocin and vasopressin, and their orthologs in other tetrapods and coelacanth, the fugu and *Tetraodon* orthologs are separated by several unrelated genes and flanked by nonsyntenic genes [23]. Likewise, most of the genes flanking the duplicated protocadherin clusters in fugu are unrelated to the conserved syntenic genes flanking the unduplicated protocadherin cluster in the elephant shark and human [24•].

Protein sequences are evolving faster in fishes

The most common fate of duplicated genes is that while one of the daughter genes continues to be under selective pressure and retains the ancestral function, the other gene diverges and becomes nonfunctional through the accumulation of deleterious mutations. Less frequently,

both daughter genes are retained owing to neofunctionalization of one of the daughter genes (through accumulation of favorable mutations), subfunctionalization of the daughter genes (whereby both accumulate complementary mutations such that the functions of the ancestral gene are now partitioned between the two genes) or a combination of both (subneofunctionalization) [25].

Following WGD in the teleost ancestor, 15–25% of duplicated genes have been retained in pairs [6,22,26••]. Analyses of the evolutionary rates of protein-coding sequences have indeed revealed an asymmetric evolutionary rate between the duplicated gene pairs [10,26••,27,28•,29], which is suggestive of neofunctionalization and/or subfunctionalization. However, what is striking is that even the genes retained in single copy are evolving faster compared to their orthologs in mammals. This feature of teleost genes was first revealed when the substitution rates of a set of 19 genes in teleost fishes and mammals were analyzed. Most of the genes were found to be evolving at a higher rate in teleost fishes than in mammals, irrespective of whether they were duplicated or singletons [30]. Subsequently, pairwise comparisons of a genome-wide set of 5800 orthologous genes in *Tetraodon*, fugu, human, and mouse indicated that the neutral mutation rate between *Tetraodon* and fugu is higher than in human and mouse [6]. It was also observed that the protein sequences between the two pufferfishes are more divergent than between the two mammals even though the two pufferfishes diverged more recently (~32 Mya) than human and mouse (~61 Mya) [6]. Furthermore, comparisons with their orthologs in the urochordate *Ciona intestinalis* showed that the average frequency of nonsynonymous mutations between *Ciona* and *Tetraodon* is higher than between *Ciona* and human [6], providing further support that protein sequences in teleosts are evolving faster than in mammals. A recent investigation of substitution rates in a strict set of singleton and duplicate genes in *Tetraodon* and fugu has provided clear evidence that the singleton genes and at least one copy of the duplicate genes in pufferfishes are evolving significantly faster than their human and mouse orthologs [26••]. Although the asymmetric evolution of the duplicated genes could be attributed to the relaxed selective pressure, it is unclear whether the accelerated substitution rate of singleton genes is also related to WGD. Comparative analysis of substitution rates of singleton and duplicated genes in teleosts and their orthologs in basal ray-finned fishes that escaped WGD and mammals should clarify this.

CNEs have diverged faster in fishes

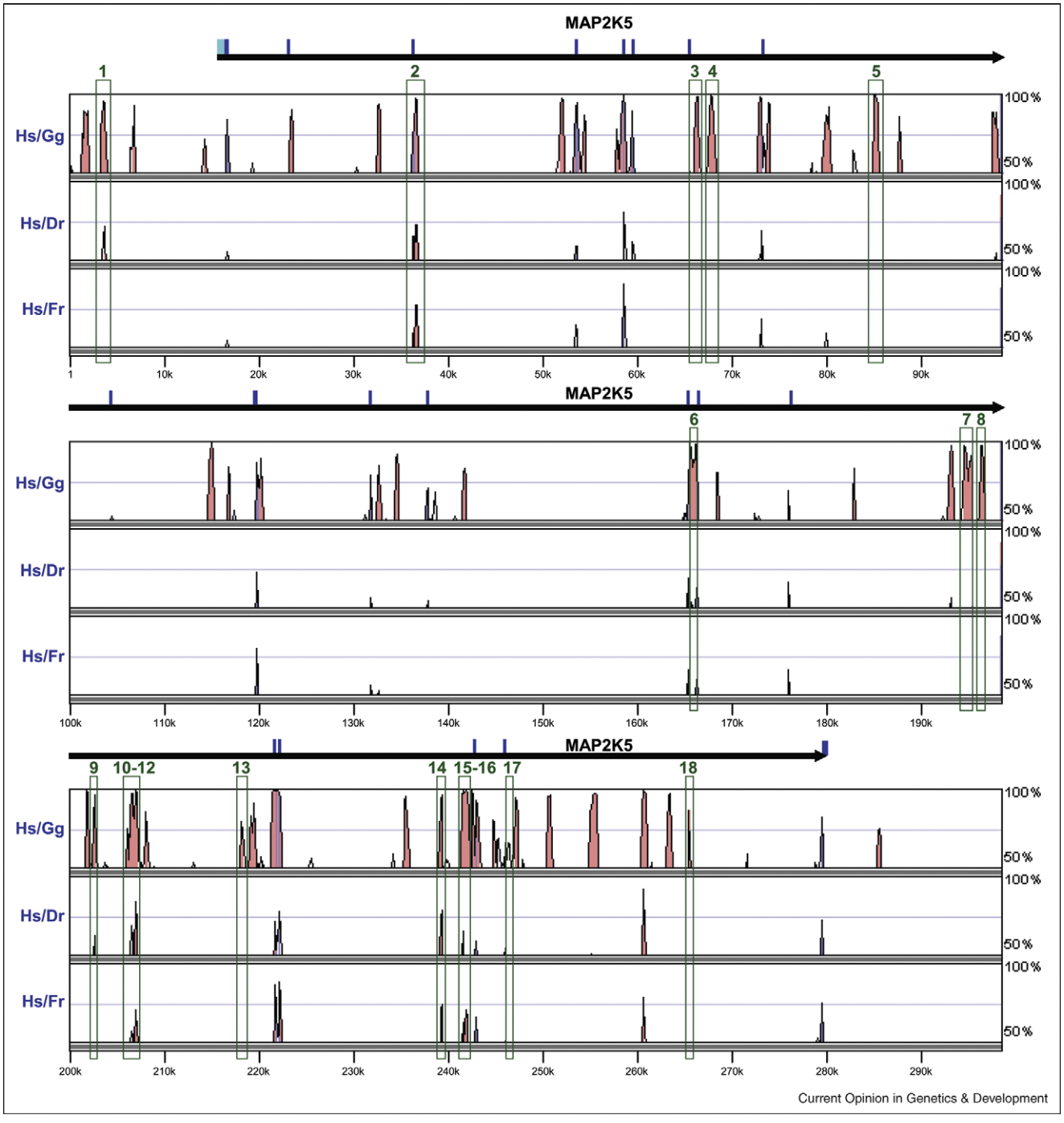
Extreme evolutionary conservation is a useful signature for identifying putative *cis*-regulatory elements in the human genome. Indeed, *in vivo* assay of noncoding elements conserved between human and distant vertebrates has indicated that many of them function as *cis*-regulatory elements directing tissue-specific expression of target genes [31–33]. Attempts to trace the evolutionary history of such elements in the human genome have indicated that they were recruited at different stages during the vertebrate evolution, with substantial numbers recruited in the bony vertebrate ancestor and the tetrapod ancestor [34••]. In fact, some conserved *cis*-regulatory elements can be traced even to cephalochordates (amphioxus [35••,36]), which are the most basal group of chordates.

Although several thousand CNEs have been identified between human and teleost fishes [31–33,34••], comparisons with more ancient vertebrates such as cartilaginous fishes have revealed that a significant number of ancient CNEs have been lost or diverged beyond recognition in teleost fishes. A large number of CNEs shared between the single HoxA cluster in human and horn shark (*Heterodontus francisci*) could not be identified in the duplicated HoxA clusters in zebrafish [37]. Interestingly, the pattern of loss is inconsistent with the duplication–degeneration–complementation model [38] which predicts that the duplicated genes retain complementary subsets of the ancestral *cis*-regulatory elements. Thus, the loss of these ancient CNEs in the zebrafish could be the result of adaptive modifications [37].

Genome-wide comparisons of CNEs in the elephant shark, human, fugu, and zebrafish have indicated that the loss of CNEs in teleosts is a genome-wide phenomenon. As compared to 4782 CNEs (≥70% identity across >100 bp) identified between the human genome and the 1.4× coverage assembly of the elephant shark genome, only 2107 and 2838 CNEs were identified between fugu–human and zebrafish–human genomes, respectively [39••]. Indeed, the divergence of ancient CNEs has been experienced by both singleton (an example shown in Figure 2) and duplicated fugu and zebrafish genes. The more recently completed genomes of medaka and stickleback contain a similar number of ancient CNEs to that in fugu and zebrafish (AP Lee, B Venkatesh, unpublished). Surprisingly, although almost all the 4782 elephant shark and human sequences are conserved in the chicken and dog genomes, only ~35% are recognizable in fugu and zebrafish. This indicates that a significant number of ancient CNEs (~3100) that are shared between cartilaginous fishes and tetrapods have been lost or diverged beyond recognition in teleost fishes.

The absence of a large number of elephant shark–human CNEs in diverse teleosts such as fugu, zebrafish, medaka, and stickleback indicates that the divergence of the CNEs began before the radiation of teleosts. The divergence might have, in fact, started in the stem ray-finned fish lineage as indicated by the pattern of CNEs in the HoxA cluster of bichir, which belongs to the most basal lineage of extant ray-finned fishes [40,41]. Although the

Figure 2



Teleost fishes have lost many ancient CNEs that are conserved in the elephant shark and tetrapods. A VISTA plot of the MLAGAN [48] alignment of human (Hs) *MAP2K5* gene with chicken (Gg), zebrafish (Dr), and fugu (Fr) orthologs showing the CNEs conserved in various vertebrates. The human sequence was used as the base sequence (*x*-axis) for the alignment. The black arrow denotes the *MAP2K5* gene with exons shown as blue boxes. It is a single copy gene in zebrafish, fugu, and other vertebrates. *y-Axis* represents percent sequence identity. CNEs were predicted using the criterion of $\geq$70% identity across $>$100 bp windows. Violet peaks represent conserved exonic sequences and pink peaks represent CNEs. Ancient CNEs conserved between the elephant shark and human (numbered 1–18) are highlighted with green boxes. The IDs of these CNEs are: 1, EH12903; 2, EH12904; 3, EH12906; 4, EH12908; 5, EH12910; 6, EH12912; 7, EH12913; 8, EH12914; 9, EH12916; 10, EH12917; 11, EH12918; 12, EH12919; 13, EH12920; 14, EH12924; 15, EH12925; 16, EH12926; 17, EH12928; 18, EH12930 [39]. All 18 CNEs are conserved in the dog genome (data not shown) while the chicken (Gg) genome has 17 CNEs (CNE 17 is missing). On the other hand, only three of them (CNEs 2, 12, and 14) are conserved in both zebrafish and fugu. One (CNE 1) is conserved in zebrafish but lost (deleted or diverged beyond recognition as a CNE) in fugu, and another one (CNE 16) is conserved in fugu but lost in zebrafish. The remaining 13 (CNEs 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, and 18) have been lost in both fugu and zebrafish.

unduplicated HoxA cluster in bichir has lost some of the CNEs shared by the horn shark and human, it has retained some that have been lost in the duplicated HoxA clusters of zebrafish and fugu. In addition, bichir has acquired some new CNEs that are conserved in teleost fishes [42]. Therefore, it appears that the divergence of ancient CNEs began in the stem ray-finned fish lineage and continued during the evolution of teleost fishes.

Conclusions

The teleost fish genomes have experienced a higher rate of gene-linkage disruption and chromosomal rearrangements compared to mammals. The protein-coding sequences in teleost fish genomes are evolving faster than in mammals irrespective of the duplication status of the genes. However, owing to a paucity of protein-coding sequence data from basal ray-finned fishes, it is unclear whether the higher evolutionary rate is unique to teleost fishes or common to all ray-finned fishes. A significant number of ancient CNEs (putative *cis*-regulatory elements) that are shared between cartilaginous fishes and tetrapods have evolved fast and diverged beyond recognition in teleost fishes. The limited data available from basal ray-finned fishes suggest that the divergence of ancient CNEs began in the stem ray-finned fish lineage well before the WGD. Sequencing and comparative analysis of whole-genome sequences from basal ray-finned fishes such as bichir and bowfin that diverged before the WGD should help to verify this hypothesis. The rapid changes in the genome organization, protein-coding sequences, and CNEs in teleost fishes all indicate that their genomes are evolving faster compared to those of mammals and the basal cartilaginous fishes.

The fast evolving protein sequences and the divergent CNEs in teleost fish genomes may have important implications for the morphological diversity and rapid speciation of teleost fishes. The higher neutral mutation rate in teleost protein-coding sequences offers higher chances for selection to act and retain favorable mutations, thereby accelerating the process of neofunctionalization of duplicated and singleton genes. Given that nearly 80% of genes are retained as singletons in teleosts after the WGD, the fast evolving singleton genes might have, in fact, played an important role in the acquisition of evolutionary novelties. Recent studies have indeed shown that positive selection has acted equally on both duplicated and unduplicated genes in teleosts and other vertebrates [43••]. Thus, many of the divergent protein sequences in teleosts may be associated with adaptive evolution. Although the significance of CNEs in vertebrate genomes has not been fully understood, it is hypothesized that most of them, particularly those conserved between distant vertebrates, are likely to be functional elements that are under purifying selection. The functions of only a couple of hundred CNEs have been tested so far, and the results indicate that many of them function as *cis*-regulatory elements directing tissue-specific or developmental stage-specific expression of target genes [31,33]. The divergence or loss of such ancient CNEs in teleosts can potentially alter the regulation of genes resulting in loss or gain of function in a specific cell-type or developmental stage. Such changes in the regulation of genes can result in genetic isolation between populations, leading ultimately to speciation. The altered regulation of genes also has the potential to give rise to marked differences in the morphology, physiology, and behavior between species [44]. Thus, the fast evolving protein-coding sequences and the divergent CNEs might have together played a key role in the diversification and evolutionary success of teleost fishes.

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