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Transcription of Dynein Heavy Chain 9 (*DNAH9*) in *Paralichthys olivaceus*: Insights into the Evolution of the *DNAH9* Gene

Zhenwei Wang, Conghui Liu, Jingjing Niu, Fan Yang, Xiaolong Wu and Jie Qi*
Ministry of Education Key Laboratory of Marine Genetics and Breeding, College of Marine Life Sciences, Ocean University of China, 5 Yushan Road, Qingdao, 266003, China

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Authors' Contribution
JQ and ZW conceived and designed the study. ZW, CL and ZW generated and the sequencing data. JN completed to comparison of DNAH9 genomic sequence and transcripts sequence. XW completed phylogenetic trees. ZW and FY wrote the article.

Key words
DNAH9*, evolution, duplication–degeneration–complementation, *P. olivaceus

ABSTRACT

As induced by the loss of prologues or irrelevant parts added in sequences, gene duplicates affect evolution. Following fish specific genome duplication (FSGD), gene duplicates undergo divergent pathways in gene evolution. Our aim is to map the evolution process of *DNAH9* duplication in *Paralichthys olivaceus* (*P. olivaceus*) after FSGD to gain an insight into the evolution pathway in teleostes. In this study, two duplicates (*DNAH9A* and *DNAH9B*) and two transcripts (*dnah9a and dnah9b*) were identified in *P. olivaceus*. A comparison of each genomic duplicate with each transcript *DNAH9B* could transcribe a complete transcript, *dnah9b*, but *DNAH9A* was necessary to co-transcribe *DNAH9B* with *dnah9a*. From phylogenetic trees, the *DNAH9* type of *P. olivaceus* shared the same cluster with all ray-finned fishes. However, *DNAH9* duplicates only occurred in *P. olivaceus* and *Cynoglossus semilaevis*. In conclusion, the duplication of *P. olivaceus DNAH9* occurred during FSGD, and the duplicates showed a divergent evolution path, undergoing a period of duplication–degeneration–complementation and then obtaining a new function.

INTRODUCTION

In the path of organic evolution, gene and genome duplication are thought to represent an irreplaceable part and a main source of evolution (Tostivint *et al*., 2013). In 1970, Ohno proposed that vertebrates experience two rounds of whole genome duplication (Ohno, 1970; Kasahara, 2013). Numerous reports have shown that ray-finned fish experience another round of genome duplication, namely, fish specific genome duplication (FSGD), which occurred 253 million to 404 million years ago after splitting from the tetrapod lineage (Taylor *et al*., 2003; Meyer and Van de Peer, 2005; Kim *et al*., 2008).

Three main steps occur from gene duplication for evolutionary preservation (Innan and Kondrashov, 2010). The first step is duplication, followed by the fixation phase. At this stage, certain duplicates become lost or suffer pseudogenization (Khaitovich *et al*., 2006). After selection in the second step, the final step occurs. Copies are stably maintained in the preservation phase. Although some of the exons are replaced, deleted, and/or repeated, gene changes only occur in a threshold to maintain a stable situation (Lambert *et al*., 2014). Finally, the surviving duplicates undergo different evolutionarily paths, such as subfunctionalization, neofunctionalization, or nonfunctionalization (Amores *et al*., 1998; He and Zhang, 2005; Kim *et al*., 2015).

Axonemal dynein heavy chain 9 (*dnah9*) is encoded by the *DNAH9* gene (Bartoloni *et al*., 2001). In particular, *dnah9* performs an irreplaceable function in cilia movement, which is related to dipleurogenesis occurring in embryonic development (Burdine and Schier, 2000; Bisgrove and Yost, 2001). Certain discoveries have shown the gradual evolution of flatfish asymmetry (Janvier, 2008). Therefore, *P. olivaceus* with external asymmetry evidently represents a unique status in evolution. Research on *DNAH9* gene evolution in *P. olivaceus* is helpful to further understand the *DNAH9* evolution in ray-finned fishes.

METHOD

Data analyses

Both *dnah9a* and *dnah9b* sequences were searched from the transcriptome of *P. olivaceus.* The *dnah9* of other species was found from the NCBI website (http://www.ncbi.nlm.nih.gov) (*Alligator sinensis*: XM_014519183.1; *Astyanax mexicanus*: XM_007257249.1; *Chelonia mydas*: XM_007072572.1; *Clupea harengus*: XM_012814636.1; *Cynoglossus semilaevis*: XM_008314866.1; *Esox lucius*: XM_010891866.1; *Fundulus heteroclitus*: XM_012855756.1; *Larimichthys crocea*:

* Corresponding author: qijie@ouc.edu.cn
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Abbreviations used: FSGD, fish specific genome duplication; DDC, duplication–degeneration–complementation.

XM_010731533.1; *Latimeria chalumnae*: XM_014494523.1; *Maylandia zebra*: XM_004569683.1; *Oreochromis niloticus*: XM_013273578.1; *Poecilia formosa*: XM_007562088.1; *Pundamilia nyererei*: XM_013913056.1; *Stegastes partitus*: XM_008278388.1; *Takifugu rubripes*: XM_011618792.1; *Xenopus tropicalis*: XM_012952760.1; *Microtus ochrogaster*: XM_005349858.1; *Zonotrichia albicollis*: XM_014272027.1; *Homo sapiens*: NM_001372.3; and *Anolis carolinensis*: XM_008104263.1). *DNAH9A* and *DNAH9B* sequences in *P. olivaceus* and *C. semilaevis* were searched from the genomic library, and other species genomic sequences of *DNAH9* were obtained from Ensemble (http://www.ensembl.org/index.html) and NBCI (*Coelacanth*: ENSLACG00000003371*; Danio rerio:* ENSDARG00000103383; *Fugu rubripes*: ENSTRUG00000000064; *Lampetra japonica*: ENSPMAG00000005143; *Maylandia zebra*: NW_004532118.1; and *Xiphophorus maculatus:* NW_005372467.1). The sequence of *DNAH9* (including *DNAH9a* and *DNAH9b*) and *dnah9* (including *dnah9a* and *dnah9b*) were compared in the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic analyses

The phylogenetic tree based on transcript sequence was constructed by MAGA6.0. The *DNAH9* genomic sequences were aligned in Clustal W, and the phylogenetic tree based on genomic sequence was constructed by MrBayes.

RESULTS

Analysis of DNAH9 duplicate sequence in P. olivaceus

In this research, we found two duplicates of *DNAH9* (*DNAH9A* and *DNAH9B* with lengths of 54678 and 136217 bp, respectively) in the genome and two transcripts [*dnah9a* (14796 bp) and *dnah9b* (13368 bp)] from the transcriptome of *P. olivaceus.* After comparing each transcript (*dnah9a* and *dnah9b*) with each genomic duplicate (*DNAH9A* and *DNAH9B*) (Fig. 1A), 96 exons were found in *DNAH9A*, and 103 exons were detected in *DNAH9B. DNAH9B* transcribed a complete transcript, that was*dnah9b* (Fig. 1A). By contrast, *DNAH9A* failed to transcribe *dnah9a* because of exon deletion (Fig. 1A). The segment insertion, deletion, and repeat were observed in *DNAH9A*. Furthermore, *DNAH9A* may undergo fragment insertion, loss, or repeat after *DNAH9* duplication and cannot be transcribed alone (Fig. 1B).

Evolutionary relationship with other species on genomic sequence

Comparison of genome sequences among several fishes searched from Ensemble and NCBI GenBank showed two duplicates existing in the genome of *C. semilaevis* and *P. olivaceus*, whereas other fish species displayed only one duplicate (Fig. 2). As shown in Figure 2, the evolutionary relationship of *DNAH9* with *C. semilaevis* and *P. olivaceus* was divided into two groups, which differs from its closer species relative.

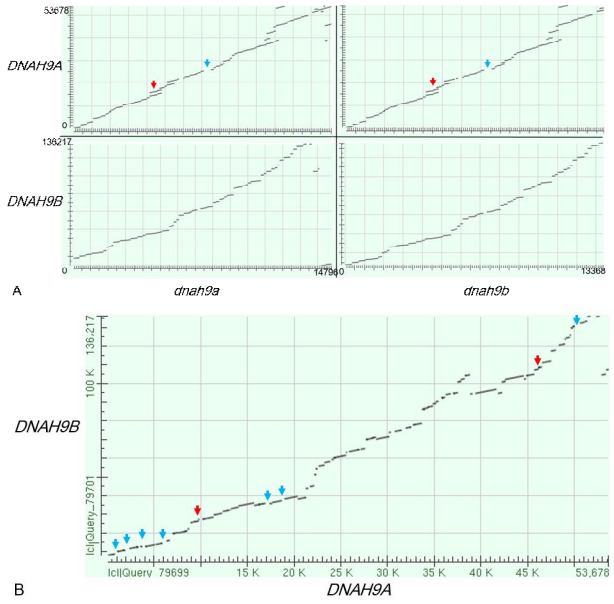

Fig. 1. Comparison of *DNAH9* genomic sequence and transcripts sequence. (A) Comparison of *DNAH9* duplicates with transcripts *dnah9a* and *dnah9b*. (B) Comparison of the two genomic sequences; the red arrow refers to one of the segment repeats, whereas the blue arrow refers to one of the segment deletions.

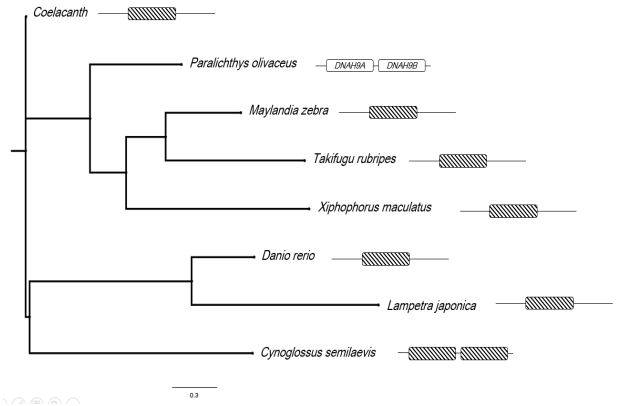

Fig. 2. Phylogenetic trees based on *DNAH9* genomic sequence in *P. olivaceus* and other chordates.

Phylogenetic analysis based on DNAH9 transcript sequence

To evaluate the evolutionary relationships of *dnah9* between *P. olivaceus* and other vertebrates, a genealogical tree was constructed on the basis of putative amino acid sequences by using MEGA6.0 with neighbor-joining method. Both *dnah9a* and *dnah9b* were aligned with other species and used to establish the phylogenetic tree. Similar results are shown in Figure 3.

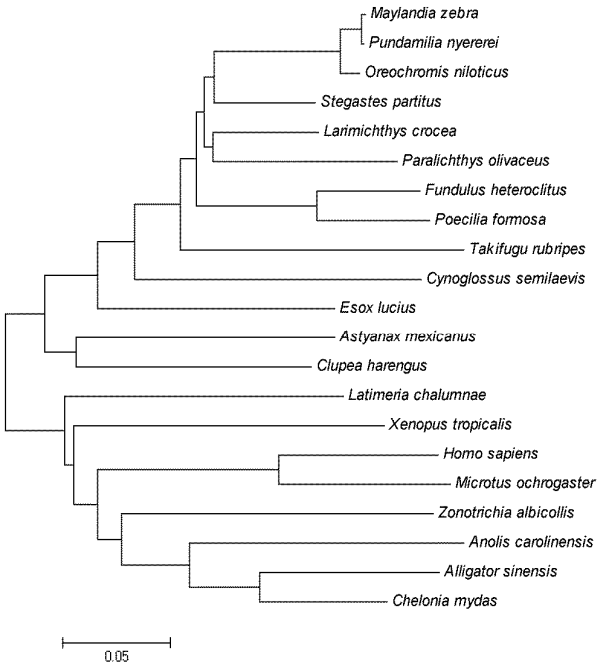

Fig. 3. Phylogenetic trees based on *DNAH9* transcripts of *P. olivaceus* and other vertebrates.

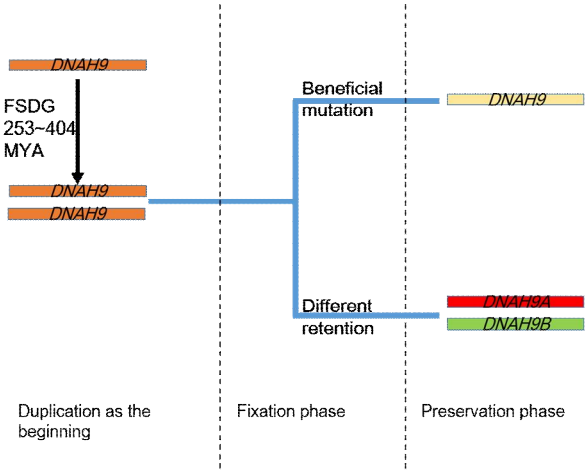

Fig. 4. *DNAH9* evolution model after FSGD.

From the phylogenetic tree, the *DNAH9* in *P. olivaceus* shared the same cluster with all ray-finned fish. As a significant species representing the transition phase from aquatic to terrestrial animals, *L. chalumnae* shared the same branch with the tetrapod. This observation suggested that the duplication of *DNAH9* occurred in the FSGD event after separation from the tetrapod.

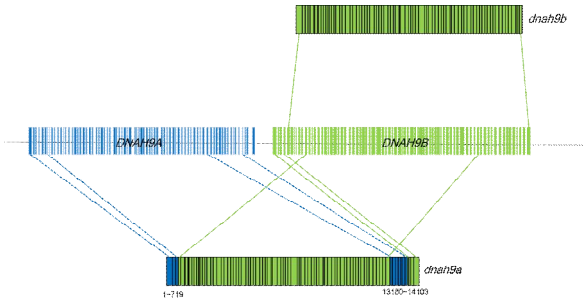

Fig. 5. Translation model of the two transcripts, *dnah9a* and *dnah9b*, of *P. olivaceus*.

DISCUSSION

Different evolution phases of DNAH9 in P. olivaceus *with other actinopterygii*

Analysis of the *DNAH9* genome sequence in teleost indicated that the duplication of *P. olivaceus* and *C. semilaevis DNAH9* occurred upon FSGD.

Three steps are involved in analyzing the rate of evolution after FSGD. *DNAH9* duplicates in the FSGD, and then the daughter duplicates co-exist in the organism, thereby representing the beginning of gene evolution. The next step is the fixation phase. Some ray-finned fishes preferentially diverged toward the evolution pathway of beneficial mutation, in which one duplicate is abandoned after duplication. Meanwhile, *P. olivaceus* and *C. semilaevis* tend to take another pathway, that is, differential retention, to preserve the duplicates. The final step is the preservation phase. Duplicates in organisms experience limited changes in a control scope. Although most duplicated genes tend to undergo beneficial mutation after FSGD, 3.3% to 7.2% of duplicated genes survive during evolution. These genes can produce certain new characters (Krause and Pestka, 2015).

Transcription mode of DNAH9 duplicates in P. olivaceus

Two homologous transcripts, *dnah9a* and *dnah9b*, exist in *P. olivaceus*. In particular, *dnah9b* can be completely transcribed by *DNAH9B*. However, some portions of *dnah9a* with identities 100% with *DNAH9A* and 83% with *DNAH9B* were more highly identified with *DNAH9A* and other portions with identities 98% with *DNAH9A* and 100% with *DNAH9B* preferred *DNAH9B*.

As such, we predicted that *dnah9a* is co-transcribed by *DNAH9A* and *DNAH9B*. The results also indicated that *DNAH9A* may perform a coordinate transcription role.

Two assumptions can explain this situation. First, *DNAH9A* underwent duplication–degeneration–complementation (DDC) after FSGD. DDC is a temporary status between gene duplication and subfunctionalization (Amores *et al.*, 1998; Wang *et al.*, 2010). After duplication, each duplicate suffers from the deleterious mutant, and neither duplicate can perform the ancestral function. For instance, both *DNAH9A* and *DNAH9B* cannot completely transcribe the two transcripts alone. Therefore, the duplicates must work together to be functional. As described in Fig. 5, *DNAH9A* transcribes certain parts of *dnah9a* and other parts of *dnah9a* derived from *DNAH9B* to perform the ancestral task. The second assumption indicates that after FSGD, the original *DNAH9* and its daughter duplicates will form a new fusion gene. The two transcripts, *dnah*9a and *dnah9b*, are different isoforms of fusion *DNAH9*.

Results showed the evolutionary relationship of *DNAH9* in *P. olivaceus* and *C. semilaevis* differs from other species. It might be the reason that only its *DNAH9* suffered the subfunctionliazation or neofunctionliazation while other species went through the nonfunctionliazation which led the duplicates disappeared. In this sense, *DNAH9* duplicates may perform an irreplaceable function in the character formation of *P. olivaceus* and *C. semilaevis*, which exhibit a clear external left–right asymmetry with both eyes localized on a single side of the body.

CONCLUSION

Gene duplication plays an important role in evolution (Mahmood *et al.*, 2016). Divergent duplication evolution in ray-finned fishes occurred after FSGD. Some fishes suffered beneficial mutation, whereas others preferentially preserved the duplicates, such as the *DNAH9* duplicates in *P. olivaceus* and *C. semilaevis*. Both of these duplicates were preserved. In *P. olivaceus*, one of the duplicates, namely, *DNAH9A*, underwent segment deletions and repeats in the preservation phase and co-transcribed *dnah9a* with *DNAH9B*. The special evolutionary pathway of *DNAH9A* did not only contribute to further understanding the gene duplicate evolution but also recognized a new element for the asymmetry of flatfish.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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