Comparative Analysis of Mitochondrial Genome in Fishes

Dilip Kumar Bej and Bishwajeet Panda

Department of Zoology, Fakir Mohan Autonomous College, Balasore, Odisha, India

Corresponding author: Department of Zoology, Fakir Mohan Autonomous College, Balasore, Odisha, India. Email: dillipkumarbej@gmail.com

Abstract

Mitochondrial genome is circular double stranded DNA molecule in most of the eukaryotic organism which is ubiquitously present in all cells as it associated with production of energy. The number of complete mitochondrial genomes sequenced has been increasing day by day. Till date, the complete mitochondrial genome more than 2000 fish species can be retrieved from the NCBI GenBank. Like other vertebrates, fish mitochondrial genome contains 37 genes which include 22 tRNAs, 2 rRNAs (12S & 16S), 13 protein coding genes and one noncoding regions (Control region). The length of fish mitochondrial genome varies based on the size of noncoding region (CR). The complete mitochondrial genome sequence length varies from species to species. This comparison of genome sequences envisage the variations are mostly in the control region as it is highly evolved than other genes of mitochondria. Its mutation rate is very high. Therefore more numbers of indels and intergenic sequences are found. It may be due to the genetic drift, relaxed functional constraint and reduced selection pressure in the evolution of fish mitogenomes.

Keywords: Mitochondrion, Mitochondrial genome, Control region, Protein-coding genes

Introduction

Origin and Structure of mitochondrial genome

The mitochondrial genome are believed to be originated from a symbiotic association between α-proteobacteria with earlier prokaryotic cell representing an endosymbiotic theory which later on gives rise to an eubacterium which comprises an eukaryotic nucleus and cytoplasmic organelles. This mutually benefited relationship gives rise to eukaryotic radiation as an organelle like mitochondrion.

Mitochondrion has two membranes, an outer membrane having mainly permeability properties and an inner membrane as electron transport and oxidative phosphorylation. The inner space, called matrix, contains several metabolic enzymes, the mitochondrial DNA that serves as genetic apparatus for its replication and expression. The electrons flow through the electron transport system that result in fall of the free energy that used to pump the protons (H^+) from the matrix to the intermembrane space. Thus, it establishes an electrochemical gradient of proton ion across the membrane which promotes the adenosine 5'-triphosphate (ATP) synthesis catalyze by enzyme ATP synthase through the reverse flow of protons from perichondrial space to matrix [1].

Mitochondrial Genome Organization in Vertebrates

Mitochondrial genome is circular double stranded DNA molecule in most of the eukaryotic organism. The mitochondrial genome has vary in length as in *Plasmodium falciparum* a protozoan parasite is having 6 kb long, whereas in musk melon a plant has a long 2500 kb of mitochondrial genome. In all organisms, this mtgenome encodes very few gene products that are essential for the oxidative phosphorylation. All other proteins that required for function of mitochondria are encoded by nuclear DNA and transported into the various organelles. In vertebrates, the size of the mitochondrial genome is about 16 kb, while it is variable within some invertebrate groups [2]. It contains two rRNA genes (12S & 16S), 13 protein coding genes and 22 tRNAs.

The main regulatory region so called control region of mtgenome about 1 kb in mammal. It is called 'D loop', because the nascent H strand creates a triple-stranded structure with the displacement of the old strand during initiation of replication. This region contains the origin of replication of the H strand and two promoters, the heavy-strand promoter (HSP) and the light-strand promoter (LSP). These 13 protein coding genes present in mitochondrial genomes are used absolutely for the production of ATP through oxidative phosphorylation [3].

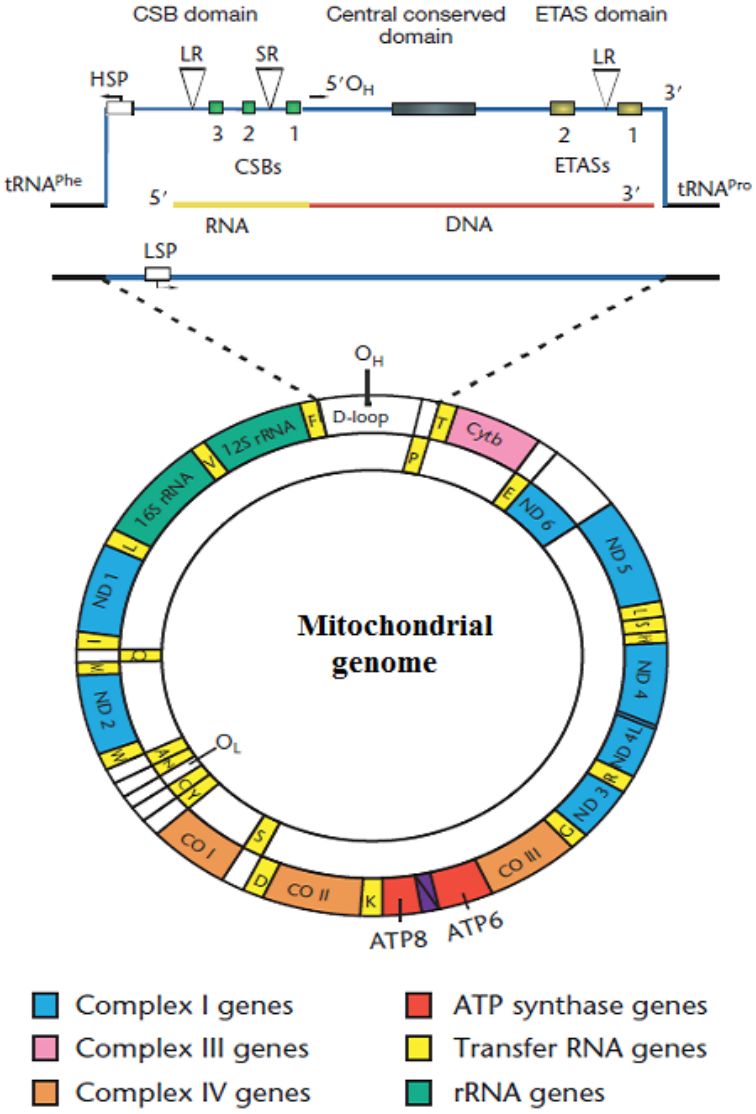

Fig. 2. Gene organization in mitochondrial genome with depiction of various gene contents in respective heavy chain and light chain.

Mitochondrial genome in fishes

Complete mitochondrial genome sequences reported in fishes have the same basic structure and gene organization observed in other vertebrate species, including humans [4-5]. Since the first complete mitochondrial genome sequenced in 1981[6], the number of complete mitochondrial genomes sequenced has been accumulating at an increasing rate. Till date, the complete mitochondrial genome more than 2000 fish species can be retrieved from the NCBI GenBank (http://www.ncbi.nlm.nih.gov). Like other vertebrates, fish mitochondrial genome contains 37 genes which include 22 tRNAs, 2 rRNAs (12S & 16S), 13 protein coding genes and one noncoding regions (Control region). The length of fish

mitochondrial genome varies based on the size of noncoding region (CR). The complete mitochondrial genome sequence length varies from species to species [7-8]. In Common carp 16, 575 bp [9], Zebra fish 16, 596 bp [10], Grass carp 16, 609 bp [11], Hook snout carp 16, 611 bp [12], Mekong Giant cat fish 16, 533 bp [13], Helmet cat fish16, 539 bp [14], and in Sea bass 16, 535 bp [15].

Most of these genes are encoded by the heavy (H) strand, which includes two rRNA genes, 14 tRNA genes, and 12 protein genes. The light (L) strand encodes the rest of genes. The same genes are also encoded, respectively, by the H- and L-strand of the mtDNAs of other vertebrates. The coding regions on both strands of carp mtDNA have about the same length as those found in other vertebrates. The majority of the protein genes and the two rRNA genes of the carp mitochondrial genome are interposed by at least one tRNA gene. These tRNA genes are thought to act as a recognition signal for mitochondrial RNA processing [16].

Mitochondrial Genome of Cyprinids

In Cyprinids, the gene rearrangement of mitochondrial genome is similar to other ostariophysian i.e Characiformes, Siluriformes and Gymnotiformes [8]. The gene content and its organization of fish mitochondrial genomes are quite conserved. This conserved characteristic facilitates their alignment and identification. The protein coding genes, tRNAs, rRNAs and non-coding control region of some cyprinid species have been described.

1.1 Protein Coding Genes

In Zebra fish (*Danio rerio*) the ATG is the starting codon of protein coding genes with exception to *COI*, which begins with GTG. The GTG codon has also been reported to initiate prokaryotic genes [17]. Stop codons include seven TAA (*ND1*, *COI*, *ATPase 8*, *ATPase 6*, *ND4L*, *ND5*, *ND6*) and three TAG (*ND2*, *COIII*, *ND3*). The *COII*, *ND4*, and *Cyt b* genes do not possess proper stop codons but it show a terminal T or TA. This condition is common among vertebrate mitochondrial genes and it appears that TAA stop codons are created via posttranscriptional polyadenylation [16]. Reading frames of two pairs of genes, *ATPase 8 – ATPase 6* and *ND4L–ND4* overlap by seven nucleotides and *ND5–ND6* overlap by four nucleotides [10].

In Common Carp (*Cyprinus carpio*) all protein coding genes use the orthodox ATG codon as the translational initiator with exception to the *COI* gene, which begins with the sequence GTG. Among the 13 protein genes encoded by carp mtDNA, six genes (*ND1*,*COI*, *ATPase 6*, *COIII*, *ND4L*, *ND5*) end

with TAA and three genes (*ND2*, *ATPase 8*, *ND3*) ends with TAG as the stop codon, whereas (*COII*, *ND4*, *ND6*, *Cyt b*) genes end with a incomplete stop codon i.e. with T or TA. The carp *ND4L* and *ND4* genes overlap by 7 bases as found for the same pair of genes in other vertebrates. The *ATPase 8* and *ATPase 6* genes of carp mitochondria overlap by 7 bases [9].

In Grass Carp (*Ctenopharyngodon idella*) the common start codon ATG in all mitochondrial protein coding genes except in the *COI* gene, which uses GTG rather than ATG. Three types of stop codons were used by the coding genes, including TAA for *ND1, COI, ATPase8, ATPase6, COIII, ND4L, ND5, ND6* and *Cyt b; TAG for ND2, ND3* and *ND4*; and an incomplete stop codon T- for *COII*, respectively. The total length of 13 mitochondrial protein-coding genes in the grass carp is 11, 394 bp, accounting for 66.6% of the complete mitochondrial genome [11].

In Hook Snout Carp (*Opsarichthyes bidens*) mitochondrial protein coding genes use ATG as the initiation codon with the only exception of *COI*, which uses GTG as the start codon. Stop codons include five TAA and four TAG. The *COII, COIII, ND4* and *Cyt b* genes possess incomplete stop codons and show a terminal T or TA. Of the 13 protein-coding genes, gene overlaps were observed between four pairs of the contiguous genes, *ATP8–ATP6, ATP6–COIII, ND4L–ND4* and *ND5–ND6* and they overlap by seven, seven, one, and four nucleotides respectively. Nucleotide composition of the *O. bidens* mtDNA protein coding genes reflects a weak bias against G on the light strand, the sense strand for all protein genes except *ND6*, for which the heavy strand serves as the sense strand [12].

1.2 Transfer and Ribosomal RNA Genes

In Common carp (*Cyprinus carpio*) the sequences complementary to the anticodons of the 22 tRNAs are similar to those of the homologous tRNAs encoded by the mtDNAs of other vertebrates. All the 22 tRNAs encoded by carp mtDNA can be folded into cloverleaf secondary structure with 7 bp in the amino acid stem, 5 bp each in the TΨC and anticodon stems, and 4 bp in the DHU stem. The tRNA genes of carp mitochondrial genome do not encode the 3' CCA terminus. Consequently, they are added post transcriptionally. In carp mtgenome, the 12S and 16S rRNA genes are, respectively, bordered by the *tRNA* ^{Phe} at the 5’ end, interspersed by the *tRNA* ^{Val} and *tRNA* ^{Leu} (UUR) gene at the 3' end. The same organizations of these two rRNA genes are also observed in other vertebrates.

All Zebra fish (*Danio rerio*) mitochondrial tRNA genes have anticodons that match the vertebrate mitochondrial genetic code. Each tRNA sequence may be folded into a cloverleaf structure with 7 bp in the aminoacyl stem, 5 bp in the TΨC and anticodon stems, and 4 bp in the DHU stem. tRNA stem regions include numerous noncomplementary and T-G base pairings, several of which are

shared with carp. Such mutations appear to accumulate in mitochondrial genes, in part because mtDNA is not subject to the process of recombination, which may facilitate elimination of deleterious mutations.

The Grass carp (*Ctenopharyngodon idella*) mitogenome has two rRNA subunits - 12S and 16S, as in other vertebrates. The two subunits are separated by *tRNA* ^{Val} and the length between two rRNA subunits was found to differ from species to species [18]. Twenty-two tRNA genes were observed in the grass carp mitogenome and their lengths ranged from 68 to 76 bp. There were overlapping nucleotides in certain adjacent tRNA genes.

1.3 The Noncoding region

In Common Carp, the D-loop region of mtDNA is about 928 bp in length and its base composition is rich in A and T (65%). Sequence analysis of vertebrate mtDNAs has revealed that the D-loop region is the most rapidly evolving part of the genome. A putative termination-associated sequence (TAS) of TACATATTAT is identified in the 3' end of the D-loop region of carp mtDNA. A putative promoter for H-strand transcription (HSP) of the sequence of ACCAAAAATCCCCAAAAAAGA is noted at 55 bases upstream of the 5' end of *tRNA* ^{phe} gene. A structure located between the *tRNA* ^{Asn} and *tRNA* ^{Cys} gene of carp mtDNA similar to that of the origin of L-strand replication of other vertebrates. This sequence has the potential to form a stable stem-loop structure. In carp, this structure consists of a stem of 11 bp, a loop of 14 bases, and a 5' flanking sequence. This structure is involved in the transition from RNA to DNA synthesis during replication initiation. The sequence of the origin of L-strand replication is well conserved in the vertebrate mtDNAs [9].

In Zebra fish the major noncoding region (control region) in mtDNA regulates replication and transcription. The primary sequence of much of the control region does not appear to be particularly important for regulatory function, as this region shows extensive variability across taxonomic groups and even among closely related species. The 950 bp zebra fish control region was much less similar to other fishes than were the coding sequences, with numerous nucleotide substitutions and insertions and deletions. Conserved sequence blocks (CSBs) 1–3, found in the 3’ end of the control region, appear to be involved in positioning RNA polymerase both for transcription and for priming replication [10].

The major noncoding region, D-loop of the grass carp (*Ctenopharyngodon idella*) mitogenome was observed 928 bp long and has three domains. The first domain is a hypervariable domain which is 246 bp long and includes a termination-associated motif sequence (TAS). Two copies of the conserved motif TACAT and its complement ATGTA in this domain can form a thermostable “hairpin” structure

for the regulation of mitochondrial gene replication (Kartavtsev et al., 2007). The second domain is a central conserved domain covering the 247–575 bp stretch. The third domain has three conserved sequence blocks (CSB- 1: 576–594 bp, CSB-2: 692–708 bp and CSB-3: 734–752 bp) and includes a microsatellite of 6 TA repeats [11].

The control region, found in the Hook Snout Carp (*Opsarichthyes bidens*) mtDNA, is about 927 nucleotides long. It is much less similar to non-cyprinid ostariophysan fishes and having numerous nucleotide substitutions and insertions and deletions. The control region has an overall nucleotide composition that is rich in A and T (A + T = 65.7%). The conserved sequence blocks, CSB I–III which are thought to be involved in positioning RNA polymerase both for transcription and for priming replication are found at the 3′ end of the control region [12].

This comparison of genome sequences envisage the variations are mostly in the control region as it is highly evolved than other genes of mitochondria. Its mutation rate is very high. Therefore more numbers of indels and intergenic sequences are found. It may be due to the genetic drift, relaxed functional constraint and reduced selection pressure in the evolution of fish mitogenome. It does not follow Mendelian inheritance and its replication and partition are not directly linked to the cell cycle so, its transmission considered to be uniparental (maternal). In contrast, the Protein coding genes have very less number of variations and it is conserved in due course of evolution. However, the sequencing of mitogenomes is boosted by high-throughput sequencing techniques. Continued expansion of mitochondrial genome databases to include both a greater number of fish species and increased representation of species from throughout their range will provide an improved basis for analysis.

2. Applications of mitochondrial genome

The mitochondrial genomes are used to resolve many taxonomic issues and identification of various species/Barcoding [19-21]. DNA barcodes is the segments of 600 bp Cytochrome oxidase (*COI*) gene that proved as a fast, efficient, and inexpensive technique to catalogue all biodiversity [22]. Mtgenome also used as a marker to study the genetic variation, deduce population structure and phylogenetic consideration [23-25].

Mitochondrial DNA sequences were assessed to evaluate the genetic variability in small marine fish *Pomatoschistus microps* [26], brown trout [27] and *Macquaria novemaculeata* [28]. In fresh water fish the genetic diversity and population structure evaluated by cytochrome *b* gene in *Channa marulius* [29], *Labeo rohita* [30], *Chitala chitala* [31] and *Cirrhinus mrigala* [32], *Cephalloscyllium umbratile* [33].

In forensic science the application of mitochondrial genome is also robust. The collection of hair, blood, semen and saliva from the crime spot that followed by PCR amplification of hypervariable region of D-loop. Mitochondrial genome has a significant contribution in ecological studies mainly in assessment of soil ecosystems and nematode biodiversity [34-35].

Limitations of mitochondrial genome

Despite many advantages, mitochondrial markers also have several challenges and pitfalls that at best complicate the data analysis, and at worst greatly limit their utility and confound their analysis. Fortunately, many of the pitfalls common to mitochondrial markers can be avoided by careful selection of genes during the analysis process [36].

(1) Biparental Inheritance

(2) Length and sequence Heteroplasmy

(3) Non neutrality

(4) Rapid lineage sorting

(5) Nuclear mitochondrial-like sequences

Conclusion

The Phylogenetic relationship is still controversial in many freshwater and marine fish species. More the mitochondrial genome will sequenced, that will add more information to the data base which helpful to comprehend the origin of mitochondrial intergenic regions and repeats on one side and the ultimate fate of the missing mitochondrial genes in the different mitogenomes. It will also helpful for resolving more phylogenetic and taxonomic issues. As more mitogenomes are characterized and combined with advance technologies like single-cell transcriptomics, it will be possible to expand insights of variety of replication, transcription, and translation processes that occurring outside vertebrate mitogenomes and to further understand the evolution of the flow of genes occurring between the mitochondrial and the nuclear genomes.

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