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Examining the Zebrafish (Danio Rerio) Mitochondrial Genome Sequence and Vertebrate Evolutionary Trends Mitochondrial DNA

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Abstract:

Mitochondrial DNA (mtDNA) transcription and replication genes, as well as genes encoding components of OxPhos complexes, were analysed for their expression in zebrafish at various times during development, beginning with the early embryo & continuing through the larval stage. Nucleotides that code for proteins, transfer RNAs, ribosomal RNA & a noncoding regulatory section compose the zebrafish (Danio rerio) mitochondrial genome, which is described in detail in this study. High early expression of structural OxPhos genes is consistent with the idea that the OxPhos system must be active early in embryogenesis, where mtDNA transcription must be activated as part of the process. High OxPhos structural gene expression at an early time point demonstrates the importance of an active OxPhos system throughout early development, as does the concept of early mtDNA transcription activation. Mitochondrial genome size in zebrafish was measured at 16,596 bp, which is very similar to the sizes of other teleost fishes (within 100 bp). It is only 18 nucleotides longer than the genome of the goldfish (Carassiusauratus) & 21 bp longer than the genome of the carp, both of which are cyprinids (Cyprinuscarpio).

Keywords: mtDNA, mitochondrial genome, DNA, zebrafish.

1. INTRODUCTION

As the OxPhos system depends so heavily on the mitochondrial genome, it has far-reaching implications for the physiology, development, and metabolism of all living things. As all vertebrates share a nearly identical number of copies of mitochondrial DNA (mtDNA) and an almost identical gene order, the molecular machinery required for mtDNA preservation and expression should likewise have been extraordinarily steady across time, it follows. The mtDNA of zebrafish is similar to the mitochondrial genomes of other animals in terms of size or gene content. Two ATP synthase subunits, three cytochrome c oxidase (Complex IV) subunits, one cytochrome c reductase (Complex III) subunit, & seven NADH dehydrogenase subunits are encoded (Complex I). The genome is circular, double-stranded, and 16596 nucleotides long [1]. (Complex V). This genome's transcription results in the production of 22 tRNAs and 2 rRNAs, which are both reliant on the nuclear genome and highlight the genome's semi-autonomous character. A numbers of transcription and replication factors that are nuclear-encoded, as well as mtDNA, are packed into mitochondrial "nucleoids" [2, 3] to facilitate mtDNA replication and expression. Nucleoid averages 100 nm in size & typically includes a single copy of mitochondrial DNA [4]. Responsible protein for mitochondrial transcription A belongs to the box family of the high mobility group & is thought to initiate mtDNA transcription (6), replication (7) & copy number determination (8,9,10) along with wrapping, bending, and unwinding mtDNA (5). DNA polymerase gamma (POL) plays an essential role in mtDNA replication, recombination & repair [11, 12]. The catalytic subunit, POLG A, a 3′5′ exonuclease, and accessory component, POLG B, which together form a 2:1 heterotrimer in humans and a heterodimer in these other mammalian species, are responsible for this enzyme's

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high fidelity [13].Several pieces of evidence [14,15] have shown that POLG A is crucial for mtDNA replication in a wide variety of animal models. Petite rho0 cells are created when the catalytic subunit of yeast POL (mitochondrial polymerase 1; MIP1) is mutated [16]. A lack of POL activity in fruit flies causes them to be weak and clumsy, to mature much more slowly than their wild-type counterparts, and to have obvious flaws in their adult vision. Homozygous deletion of the Polg gene in mice results in an extreme drop in mtDNA levels and the death of the mouse embryo in late gastrulation, before early organo- genesis has begun. Furthermore, Polg null mouse embryos are significantly smaller than wild-type embryos at E8.5 due to a severe respiratory chain deficiency. Yet homozygous Polg1 Caenorhabditis elegans mutants mature correctly & have shorter lives due to mitochondrial depletion, despite having normal morphological development.

TWINKLE, POLRMT, and TK2 are also important parts of the process of copying mtDNA. TWINKLE's structure is similar to that of the helicase/primase bacteriophage T7 gene 4 protein. By interacting with the mtSSB & TFAM proteins, which are structural components of mtDNA and prevent it from migrating, TWINKLE unwinds mtDNA during replication. The D-loop regulatory region of mitochondrial DNA in vertebrates is a transcription start site in both orientations. This creates polycistronic precursor RNAs in which each strand expresses all of the genes. The final mRNA, rRNA, and tRNA molecules all start out as fragments of these original transcripts. MtDNA transcription is done by RNA polymerase that is directed by mitochondrial DNA (POLRMT). POLRMT, the mitochondrial transcription factor A (TFAM), along with one of the two mitochondrial transcriptionally B paralogs make up the basic machinery needed and enough to start transcription at a specific promoter (TFB2M, also termed mtTFB2). Thymidine kinase (TK2) is a mitochondrial pyrimidine nucleoside kinase that acts as a catalyst the phosphorylation of deoxythymidine, deoxycytidine & deoxyuridine to form the corresponding deoxy-nucleotide 5'-monophosphates. This is part of the mitochondrial salvage route of deoxynucleotide synthesis. Both TK2 and deoxyguanosine kinase are mitochondrial deoxyribonucleoside kinases that help form dNTP pools. TK2 is in charge of the salvage pathways for pyrimidine nucleotides, and deoxyguanosine kinase is in charge of the salvage pathways for purine nucleotides. As mitochondria lack the capacity to synthesise deoxynucleotides from scratch, replication of mtDNA requires both pathways. In addition, nucleotides are constantly needed for replication of mtDNA because it occurs at all stages of the cell cycle. TK2 is essential for regulating mtDNA even in tissues that do not replicate. Mitochondrial genome mutations have deleterious effects on cellular function, progeny survival & human quality of life; consequently, their significance in animal life & embryo development is becoming increasingly recognised. To date, zebrafish (Daniorerio) have served as a good model in developmental genetics, but there has been little research addressing the "metabolism" of the mitochondrial genome in these fish. This study sought to investigate the timing of mtDNA replication & transcription in zebrafish embryos & zygotes because so little is known about these processes in vertebrate embryos & zygotes. The experiment examined mtDNA metabolism in zebrafish from the fertilisation stage all the way through adulthood. From the earliest stages of embryonic development to the latter stages of larval development, we analysed the expression of genes in the OxPhos system, genes involved in mtDNA transcription and replication & the mtDNA population as a whole. By identifying human nuclear genes associated with mitochondrial abnormalities, we identified a subset of nuclear genes in zebrafish that are orthologous to human mtDNA maintenance genes. We looked into how mtDNA RNA polymerase (polrmt), mitochondrial transcription factor A (mtTFA), and mtDNA polg1 (polg1) are expressed (tfam). Biosynthetic mitochondrial complexes were studied by dissecting their component subunits,

including succinate dehydrogenase complex subunit A, coenzyme c oxidase subunit Va, adenosine triphosphate synthase F1 alpha subunit & ubiquinol-cytochrome c reductase core protein II (atp5ab). The mitochondrial polycistronic transcript encoding the subunit 1 of the NADH-ubiquinone oxidoreductase was studied as well. The zebrafish is becoming an increasingly important animal model for studying mitochondrial metabolism and disorders because its mitochondrial activities have been substantially preserved throughout evolution.

1.1 Identification and Characterization of Genes Involved in Zebrafish Mitochondrial Biogenesis

Genes (polg1, tk2, twinkle, polrmt, or tfam) that make proteins that are important for the transcription and replication of mtDNA were studied to find out how and when embryonic development initiates mtDNA synthesis or expression-related enzyme activity. Mt-role nd1's as a gatekeeper for mitochondrial polycistronic transcripts and for nuclear-encoded components of mitochondrial respiratory complexes including ndufs4, shda, uqcrc2, cox5ab, & atp51 was also investigated. We used RT-PCR to amplify all of the zebrafish genes and found that three of them had alternative spliced isoforms (twinkle, polrmt, and tfam). All RT-PCR products had their transcript sequences directly resequenced to ensure accuracy. There is evidence of an early genome duplication in the teleost lineage [17], suggesting that as much as 20% of the D. rerio genome is composed of duplicated genes. Nonetheless, all of the genes checked in the duplicate gene search were found to be unique in the zebrafish genome.

2. REVIEW OF LITERATURE

Artuso et al. (2012) conducted a study on Mitochondrial DNA metabolism during early zebrafish growth (Daniorerio). Mitochondrial DNA (mtDNA) copy number in zebrafish drops drastically among 1 h post-fertilization and adulthood, indicating that mtDNA replication is inactive in early embryos & that, as in mammals, mtDNA replication is inactive in early embryos, mtDNA is partitioned into segments. Genes (such as the catalytic component of mtDNA polymerase & mitochondrial deoxyribonucleoside kinase) required for mtDNA replication are shown to be created later in zebrafish embryonic development, when mtDNA replication actually occurs. High early translation of structural OxPhos genes is consistent with the idea that the OxPhos system must be active at an early stage of embryogenesis, which in turn necessitates the activation of mtDNA transcription. This study is significant role in the first to describe the pattern of mtDNA inheritance in zebrafish throughout their development, and because it allows Danio rerio to be used as a model for investigating mitochondrial metabolism & disease.

Chen et al. (2012) performed Phylogenetic analyses of three sinipercid fish (Perciformes: Sinipercidae) using complete mitochondrial DNA sequences. Taxonomists have been perplexed for decades by the phylogenetic positions of the sinipercids, a group of twelve species of East Asian freshwater percoid fish. Mitochondrial genomes ranged in size from 16,496 bp in S. chuatsi to 17,002 bp in S. kneri and 16,585 bp in S. scherzeri. The 37 genes in the three mitochondrial genomes follow a similar organisation to that of mitochondrial genomes in other fish and have a considerable non-coding regulatory component. A cluster of 81 tandem repeats is located at the very end of the CSB-3 regulatory region of S. scherzeri. The three sinipercids' full mitochondrial genomes should aid in study into the evolution of both sinipercids and other vertebrates.

Whiteley et al. (2011) presented the research on Population genomics of wild & experimental zebrafish (Daniorerio). Through eco-evolutionary analyses of common laboratory models, a broader range of genotype-phenotype relationships can be elucidated. This study used to express sequence tags to examine the genetic diversity of 1832 polymorphic single nucleotide polymorphisms (SNPs) among 13 natural populations and 3 laboratory strains (ESTs). Seventy-one loci (3.4% of the total) were anomalous in their level of genetic divergence, while nine loci (0.5%) showed extraordinarily low FST values. Naturally, two further groups of laboratory strains that were genetically separate from the wildlife populations formed. Our understanding of genetic influences on phenotypic variation in a vertebrate species can be enhanced by laboratory & field study that incorporates genetic variation from a range of wild populations and the abundance of genomic data accessible for this model organism.

Wang et al. (2011) examined the research on repetitive sequences in the regulatory region of a Sichuan taimen's mitochondrial genome and the evolutionary implications for Salmonidae (Huchobleekeri). Using the use of primer walking sequence and a laborious but precise polymerase chain reaction, the mitochondrial DNA of the Sichuan taimen (Huchobleekeri) was sequenced (LA-PCR). The genome has 22 rRNA genes, 13 protein-coding genes, and 2 rRNA and 2 tRNA genes (CR). The data suggested that a T-type mononucleotide microsatellite or an 82 bp tandem repetition resided in the control area, both of which showed high levels of conservation among the three H. bleekeri samples analysed. The findings support the theory that Salmonidae evolved from freshwater animals and support the morphological and molecular assessments of evolutionary relationships among Salmonidae genera.

Catanese et al (2010) conducted a study on Scomber colias & Pacific Scomber japonicus are two different species of mackerel that have different evolutionary relationships as determined by full mitochondrial genomes. Scomber mackerels are commercially valuable, although their taxonomic identity is still up for debate. Although previous phylogenetic evidence from the analysis of imperfect mitochondrial or nuclear DNA sequences supports the distinction between Pacific Scomber japonicus and Atlantic Scomber colias as separate species, more research is necessary. S. coli, S. japonicus, and Scomber australasicus had their entire mitochondrial DNA sequenced so that researchers could learn more about the problem. This study's most important finding is that S. colias & S. japonicus may be reliably separated into their own evolutionary lineages within the Scomber cluster.

Da Fonseca et al. (2008) studied the adaptive development of the mitochondrial genome in mammals. Through oxidative phosphorylation, mitochondria generate up to 95% of the energy essential by a eukaryotic cell. By analysing amino acid sequence variation and the functional ramifications of variations in secondary and tertiary protein structures, they looked at the impact of natural selection on 12 protein-coding mitochondrial genes across 41 placental mammalian species. Several amino acids showed a wide range of features at cytochrome b's functionally relevant areas in species with particular metabolic needs. An essential component of F0, the ATP8 subunit showed the most adaptive change in its signal. High adaptive variance in projected loop areas was observed for ATP6, which plays a crucial role in rotor performance.

Antunes et al. (2005) discussed the study that uncovered a substantial number of earlier undiscovered mitochondrial pseudogenes in fish genomes. The largest repertoires of nuclear insertion transcripts of mitochondrial origin are found in human and plant genomes (numts). The

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genomes of Fugurubripes, Tetraodonnigroviridis, and Daniorerio were sequenced, and the results revealed 2, 5, & 10 recent numt integrations, respectively; all of these events took place less than 0.6 million years ago. F. rubripes, T. nigroviridis, & D. rerio all had numts older than 12.5 million million years, with 335, 336 & 471 numts older than 12.5 million years, respectively. In contrast to what was assumed for evolved species numts in humans, it appears that ancient numts preferentially insert into known or expected genes.

3. METHODOLOGY

Table 1 gives an overview of key features of mitochondrial genes in zebrafish. 13 protein-coding genes are present in the genome. These genes encode 7 subunits of the NADH-ubiquinone oxidoreductase complex (ND), one subunit of the ubiquinol-cytochrome c oxidoreductase complex (Cytb), three subunits of the cytochrome c oxidase complex (CO), and two subunits of the ATP synthase complex (Syn) (ATP). In addition to the 22 tRNA genes, the genome also contains the genes for the small (12S) and large (16S) ribosomal RNA subunits. **Figure 1** displays the amount of mitochondrial DNA copies present in zebrafish embryos.

4. RESULT

Table 1: Organization of the Mitochondrial Genome of Zebrafish

Gene/element	Strand	Position	Size	Start	Stop	3'
Control region	—	1–950	951	—	—	0
$tRNA^{phe}$	H	951–1019	69	—	—	0
12S rRNA	H	1020–1971	952	—	—	0
$tRNA^{val}$	H	1972–2042	71	—	—	0
16S rRNA	H	2043–3725	1683	—	—	0
$tRNA^{leu}$ (TAA)	H	3726–3802	77	—	—	0
ND1	H	3803–4777	975	ATG	TAA	3
$tRNA^{ile}$	H	4781–4854	74	—	—	—3
$tRNA^{gln}$	L	4852–4922	71	—	—	1
$tRNA^{met}$	H	4924–4992	69	—	—	0
ND2	H	4993–6039	1047	ATG	TAG	0
$tRNA^{trp}$	H	6038–6107	70	—	—	1
$tRNA^{ala}$	L	6109–6176	68	—	—	1
$tRNA^{asn}$	L	6178–6250	73	—	—	0

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O_L	—	6251–6282	32	—	—	0
$tRNA^{cys}$	L	6283–6354	72	—	—	—2
$tRNA^{tyr}$	L	6353–6423	71	—	—	1
COI	H	6425–7975	1551	GTG	TAA	0
$tRNA^{ser}$ (TGA)	L	7976–8046	71	—	—	2
$tRNA^{asp}$	H	8049–8117	69	—	—	12
COII	H	8130–8820	691	ATG	T. .	0
$tRNA^{lys}$	H	8821–8893	73	—	—	0
ATP8	H	8894–9058	165	ATG	TAA	—7
ATP6	H	9052–9735	684	ATG	TAA	—1
COIII	H	9735–10520	786	ATG	TAG	—1
$tRNA^{gly}$	H	10520–10589	70	—	—	0
ND3	H	10590–10940	351	ATG	TAG	—2
$tRNA^{arg}$	H	10939–11008	70	—	—	0
ND4L	H	11009–11305	297	ATG	TAA	—7
ND4	H	11299–12680	1382	ATG	TA.	0
$tRNA^{his}$	H	12681–12750	70	—	—	—1
$tRNA^{ser}$ (GCT)	H	12750–12819	70	—	—	1
$tRNA^{leu}$ (TAG)	H	12823–12895	73	—	—	1
ND5	H	12897–14717	1821	ATG	TAA	—4
ND6	L	14714–15232	519	ATG	TAA	0
$tRNA^{glu}$	L	15233–15301	69	—	—	6
Cytb	H	15308–16448	1141	ATG	T. .	0
$tRNA^{thr}$	H	16449–16520	72	—	—	5
$tRNA^{pro}$	L	16527–16596	70	—	—	0

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With the exception of COI, almost all genes encoding proteins begin with the conventional ATG start codon. There are seven TAA and three TAG codons in a stop codon. Instead of a stop codon, the COII, ND4 & Cytb genes contain a T or TA at the conclusion of their respective coding regions. This occurs frequently in vertebrate mitochondrial genes, & it suggests that post-transcriptional polyadenylation is the mechanism by which TAA stop codons are generated (Ojala et al. 1981). There is a four-nucleotide overlap between the reading frames of ND5 and ND6, and a seven-nucleotide overlap between those of ATP8 and ATP6 and ND4L and ND4. This characteristic is shared by all vertebrates, despite the fact that ATP8 & ATP6 overlap by 40-46 base pairs in mammals. Some additional genes have single or double nucleotide similarities to neighbouring tRNA genes **(Table 1).**

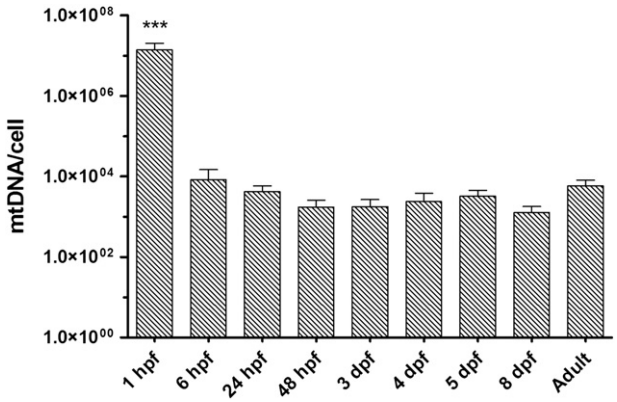

Figure 1: Copy number of mitochondrial DNA in zebrafish embryos

Mitochondrial DNA (mtDNA) quantities in developing zebrafish embryos were evaluated by polymerase chain reaction (RT-PCR). Significant reductions in mtDNA abundance were seen between the 4-cell stage (1,407 copies per cell) & the shield stage at 1 & 6 hours post-fertilization (8000 copies per cell). The levels of mtDNA in developing embryos were measured to be 4000, 1700 & 1750 copies per cell at 24, 48 & 3 days after fertilisation (hpf), respectively; after a rise to 2300 and 3100 copies per cell at 4 & 5 dpf, mtDNA decreased to 1250 copies per cell at 8 dpf. The average number of mitochondrial DNA copies in cells was 5,500 **(Figure 1).**

CONCLUSIONS

This study investigated the Vertebrate Evolutionary Patterns & the Mitochondrial DNA Sequence of the Zebrafish (DanioRerio) mitochondrial DNA. Developmental variations in the zebrafish mtDNA population were investigated in connection to the expression of genes encoding for components of OxPhos complexes & those involved in mtDNA transcription & replication. Early stimulation of mtDNA transcription & early expression of structure OxPhos genes indicate the significance of an active OxPhos system during early development. Mitochondrial transcription factor A & mtDNA-directed RNA polymerase are universally expressed in zebrafish embryos, indicating that mitochondrial transcription precedes mtDNA replication.

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