Expanding the Coding Potential of Vertebrate Mitochondrial Genomes: Lesson Learned from the Atlantic Cod

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Abstract

Vertebrate mitochondrial genomes are highly conserved in structure, gene content, and function. Most sequenced mitochondrial genomes represent bony fishes, and that of the Atlantic cod (Gadus morhua) is the best characterized among the fishes. In addition to the well-characterized 37 canonical gene products encoded by vertebrate mitochondrial genomes, new classes of gene products representing peptides and noncoding RNAs have been discovered. The Atlantic cod encodes at least two peptides (MOTS-c and humanin (HN)), two long noncoding RNAs (IncCR-L and IncCR-H), and a number of small RNAs. Here, we review recent research in the Atlantic cod focusing on putative mitochondrial-derived peptides, the mitochondrial transcriptome, and noncoding RNAs.

Keywords: Gadus morhua, long noncoding RNA, mitogenome, mitochondrial-derived peptide, mitochondrial transcriptome, mitochondrial small RNA, mtDNA

1. Introduction

The mitochondrial genome (mitogenome) is highly conserved among vertebrates [1]. All species investigated to date contain mitogenomes encoding the same 37 canonical gene products, organized in a highly similar gene order in most species. Complete mitogenome sequences have been determined from almost 5000 vertebrate species, where about 50% is represented by the bony fishes [2].

The Atlantic cod (Gadus morhua) is a benthopelagic fish in the Gadidae family, belonging to the order of Gadiformes [3, 4]. The 16.7 kb circular mitogenome was one of the first to be
Figure 1. The Atlantic cod mitochondrial genome. (A) Circular map presenting gene content and organization. The mitochondrial genome harbors 13 protein-coding genes (light blue), 2 rRNA genes (yellow), 22 tRNA genes (red), and noncoding regions (gray). CR, control region; H₁ and H₂, H-strand promoters; LSP, L-strand promoter; O₁H and O₁L, origins of heavy- and light-strand replication, respectively; HTR, heteroplasmic tandem repeat; T–P spacer, intergenic noncoding spacer. tRNA genes are indicated by the standard one-letter symbols for amino acids. All genes are H-strand encoded, except Q, A, N, C, Y, S₁, E, P, and ND6 (L-strand encoded). mtSSU and mtLSU, mitochondrial small- and large-subunit rRNA genes; ND1–ND6, NADH dehydrogenase subunit 1–6; COI–COIII, cytochrome c oxidase subunit I–III; Cyt b, cytochrome b; ATP6 and ATP8, ATPase subunit 6 and 8. (B) Schematic view of the OxPhos complexes embedded in the inner mitochondrial membrane. ATP is generated by oxidative phosphorylation. The mitochondrial genome encodes 13 of the approximately 85 subunits, belonging to complex I (blue), complex III (orange), complex IV (green), and complex V (yellow).
completely sequenced from a fish species [5–7]. Atlantic cod possesses the same mitogenome organization as most vertebrate species, including that of humans and vertebrate model systems like mouse, rat, *Xenopus*, and zebrafish (Figure 1A).

Among the canonical gene products encoded by the Atlantic cod mitogenome, 13 represent hydrophobic proteins essential for oxidative phosphorylation (OxPhos), two are ribosomal RNAs (rRNAs) of the mitochondrial ribosome, and 22 are transfer RNAs (tRNAs) necessary for mitochondrial translation. The OxPhos system consists of five large protein complexes embedded in the inner mitochondrial membrane. However, only 13 of the approximately 85 OxPhos proteins are encoded by the mitogenome (Figure 1B) [8].

Both strands (H- and L-strands) have coding potential (Figure 1A). Most mitochondrial genes are encoded by the H-strand and include the small and large subunit rRNAs (mtSSU rRNA and mtLSU rRNA), 14 tRNAs, and 12 protein-coding genes. The L-strand, however, encodes only eight tRNAs and one protein. The control region (CR), located between the genes of tRNA<sup>Pro</sup> and tRNA<sup>Phe</sup>, is the major noncoding region in the mitogenome and constitutes approximately 1000 bp in Atlantic cod [7, 9]. The CR harbors the genetic control elements for H-strand replication origin (OriH), the transcription initiation sites for H- and L-strands, as well as the displacement loop (D-loop) located between OriH and the termination associated sequence (TAS) [7, 9, 10]. Furthermore, a 30-bp spacer located between the genes of tRNA<sup>Asp</sup> and tRNA<sup>Cys</sup> contains the origin of L-strand synthesis. OriL appears functionally conserved in most vertebrates [11, 12], including the Atlantic cod [5].

Hallmarks of Atlantic cod mitogenomes are the noncoding intergenic T–P spacer, and the heteroplasmic tandem repeat (HTR) array at the 5′ domain of CR (Figure 1A). The 74-bp Atlantic cod T–P spacer [5, 13], located between the tRNA<sup>Thr</sup> and tRNA<sup>Pro</sup> genes, represents an evolutionary preserved feature present in all gadiform species [10, 13]. The T–P spacer is variable in sequence and size among gadiforms but still harbors two conserved 17-bp sequence motifs forming potential hairpin structures at the RNA level [10]. The HTR array consists of a 40-bp sequence motif usually present in two to five copies within an individual [5, 14, 15] and thus results in size heterogeneity and heteroplasmy of Atlantic cod mitogenomes. Here, we review recent developments in the characterization of Atlantic cod mitogenomes with focus on interindividual sequence variation, mitochondrial transcriptome, noncoding RNAs, and putative mitochondrial-derived peptides.

### 2. Sequence variation among Atlantic cod mitochondrial genomes

Complete mitogenome sequences have been obtained from approximately 200 specimens representing major ecotypes and geographic locations of Atlantic cod. In one study, based on SOLiD deep sequencing, we performed pooled sequencing of 44 specimens from each of the migratory northeast arctic cod (NA) and the stationary Norwegian coastal cod (NC) [16]. The sequencing represented more than 1100 times mitogenome coverage of each ecotype and 25 times coverage of each individual. We found a total of 365 SNP loci in the dataset, where 121 SNPs were shared between the ecotypes. One hundred fifty-one SNPs and ninety-three SNPs...
were specific to NA and NC cod, respectively. From the dataset we determined the mitochondrial substitution rate to be 14 times higher compared to that of the nuclear genome [16, 17].

More recently we analyzed 156 Atlantic cod mitogenomes at the individual level [18], including 32 specimens previously reported by Carr and Marshall [19]. We found 1034 SNPs in total among the sequences, which were not evenly distributed throughout the mitogenome. The ND2 gene (Complex I) and the COII gene (Complex IV) were the least and most conserved, respectively, among the protein-coding genes. Furthermore, rRNA and tRNA genes showed a significantly lower density of overall SNPs per site compared to protein-coding genes. Thus, the Atlantic cod mitogenome follows a similar pattern of conservation as seen for other vertebrates like zebrafish and human [20–23] and corroborates the observation that mutation rate constrains in vertebrate mitogenomes appear linked to the position of genes in relation to OriH and OriL [24, 25].

The noncoding regions of the Atlantic cod mitogenome showed a mosaic pattern of sequence conservation. Whereas the OriL and the central domain of CR were almost invariant among specimens, the T–P spacer and 5′ domain of CR contain significant sequence variation [7, 10, 13, 18]. The 74-bp T–P spacer was found to contain 16 variable sites and 26 haplotypes among 225 specimens assessed, including a 29-bp sequence duplication in three individuals [10]. Similarly, the 5′ domain of CR was the most variable region within the mitochondrial genome (more than three times that of average substitution rate). The elevated sequence variation was due to hot-spot substitution sites, homopolymeric heterogeneity, and the HTR array [18].

3. Mitochondrial-derived peptides

Vertebrate mitogenomes have the potential of encoding several short peptides (mitochondrial-derived peptides (MDPs)) [26–28]. The best characterized peptides among the MDPs are MOTS-c and humanin (HN). Genes coding for MOTS-c and HN are found as small open reading frames within the mitochondrial small subunit (mtSSU) and large subunit (mtLSU) ribosomal DNA, respectively [29, 30]. Studies in mammals indicate that MDPs are circulating signaling molecules with a number of proposed roles. While HN is involved in cellular stress resistance, apoptosis, and metabolism [29, 31–34], MOTS-c apparently represents an MDP hormone that regulates metabolic homeostasis and insulin sensitivity [30, 35].

The Atlantic cod open reading frames encoding MOTS-c and HN were identified at the exact same locations as in human, within the domain 3′M and domain IV of the mtLSU rRNA and mtSSU rRNA, respectively (Figure 2A and B). Comparative analysis revealed MOTS-c and HN to be invariant among Atlantic cod specimens [18] and well conserved between Atlantic cod and human (Figure 2C). Here, 8 of 16 amino acid residues in MOTS-c and 13 of 21 amino acid residues of HN were shared. Furthermore, when comparing gadiform species representing seven diverse families, we noted 10 of 16 and 15 of 21 amino acid residues to be shared in MOTS-c and HN, respectively (Figure 2C). The conserved features seen between gadiform species and human suggest related MDP functions.
Figure 2. Putative mitochondrial-derived peptides in Atlantic cod. (A) Secondary structure diagram of the Atlantic cod mtSSU rRNA domain 3'M coding for the putative MOTS-c peptide (red letters). (B) Secondary structure diagram of the Atlantic cod mtLSU rRNA domain IV coding for the putative HN peptide (red letters). (C) Alignment of MDP (MOTS-c and HN) sequences from seven gadiform species representing different families (gm, Gadus morhua, Gadidae, HG514359; Li, Lota lota, Lotidae, AP004412; mm, Merluccius merluccius, Merluccidae, FR751402; Ec, Enchelyopus cimbrius, Phycidae, AJ315624 and FJ215015; tm, Trachyrincus murrayi, Macrouridae, AP008990; Bn, Bregmaceros nectabantus, Bregmacerotidae, AP004409; Pj, Physiculus japonicus, Moridae, AP004409) and human (Hs, Homo sapiens, NC_012920). Stars above and below the alignment represent conserved residues among gadiforms and between gadiforms and human, respectively.
4. Mitochondrial transcriptome

Vertebrate mitochondrial transcriptomes have mainly been studied in human cells and tissues [36, 37]. Mature mitochondrial RNAs are generated from three polycistronic transcripts initiated within CR from two H-strand promoters (HSP$_1$ and HSP$_2$) and a single L-strand promoter (LSP) (Figure 3A) [36, 38–40]. The HSP$_1$-specific transcript is highly abundant and generates mtSSU rRNA, mtLSU rRNA, as well as tRNA$^{Val}$ and tRNA$^{Phe}$ [41, 42]. HSP$_1$-specific tRNAs have recently been proposed to perform a second role as a mitochondrial RNA, substituting the lacking 5S RNA in vertebrate mitochondrial ribosomes [43, 44]. While tRNA$^{Val}$ appears associated with the mitochondrial ribosomes in human and rat, tRNA$^{Phe}$ has been identified in porcine and bovine [45].

Ten H-strand-specific mRNAs are posttranscriptionally processed from the HSP$_2$ transcript, together with 13 tRNAs and the two rRNAs (Figure 3A) [46]. Most HSP$_2$ mRNAs are monocistronic, but two of the mRNAs are bicistronic (ND4/4 L mRNA and ATPase8/6 mRNA). Finally, the L-strand-specific transcript gives rise to the ND6 mRNA and eight tRNAs (Figure 3A).

4.1. Atlantic cod mitochondrial mRNAs

Similar to that of human cells, 11 mature mRNAs were readily expressed from the Atlantic cod mitogenome [47]. There are, however, some minor differences in mitochondrial mRNA maturation and modification between human and Atlantic cod. Mapping of the 5’ ends in mitochondrial mRNAs by pyrosequencing revealed that 10 of the 11 mRNAs contain no, or very short (1–2 nt), 5’ untranslated regions (UTRs) [47]. The only exception is the 5’ UTR of the COII mRNA, which contained a short hairpin structure. In Atlantic cod and all other Gadidae species, this hairpin structure is capped by a GAAA tetra-loop (Figure 3B) [47]. GAAA tetra-loops are known to frequently participate in long-range RNA:RNA tertiary interactions [48].

Most Atlantic cod mRNAs lack 3’ UTRs, but the COI mRNA has a 3’ UTR of 76 nt corresponding to the complete mirror sequence of tRNA$^{Ser(UCN)}$ (Figure 3B) [47]. A very similar 3’ UTR (72 nt) has been reported in the human COI mRNA [49], indicating a conserved role in vertebrates. The 3’ UTR of the ND5 mRNA is highly variable in length in vertebrates but is lacking completely in Atlantic cod [40, 47]. However, the closely related Gadidae species Pollachius virens (Saithe) contains an ND5 mRNA 3’ UTR of 16 nucleotides [47]. In humans, mitochondrial mRNAs contain short polyA tails of 40–50 adenosines at their 3’ ends [40, 45]. PolyA tails were identified in all mRNAs, except for ND6 mRNA [40], and seven UAA termination codons were created in the human mitochondria by polyA posttranscriptional editing [50]. Similarly, all mitochondrial mRNAs (except the ND6 mRNA) were found to be polyadenylated in Atlantic cod, and six UAA termination codons were generated by polyA addition [47].

4.2. Atlantic cod mitochondrial structural RNAs

The 22 mitochondrial tRNAs were found to be highly conserved in Atlantic cod, both in structure and sequence [5, 18], and some tRNAs (tRNA$^{Ile}$, tRNA$^{Ser(UCN)}$, tRNA$^{Ser(AGY)}$, and tRNA$^{Cys}$)
were invariant in the 200 specimens investigated. SOLiD deep sequencing confirmed a non-template CCA addition at the 3′ ends of tRNAs (our unpublished results). Thus, mitochondrial tRNA processing and probably modification are highly similar in human and Atlantic cod [47].

Figure 3. The mitochondrial transcriptome in Atlantic cod. (A) Schematic map of mitochondrial ribosomal RNA, messenger RNA, and long noncoding RNA generated from HSP1, HSP2, and LSP transcripts. mtSSU rRNA and mtLSU rRNA, mitochondrial small- and large-subunit ribosomal RNA (yellow boxes); ND1, ND2, ND3, ND4L/ND4, ND5, and ND6, NADH dehydrogenase subunit mRNAs; COI, COII, and COIII, cytochrome c oxidase subunit mRNAs; A8/ A6, ATPase subunit bicistronic mRNA; Cyt B, cytochrome b mRNA (all mRNAs indicated by blue boxes); IncCR-H and IncCR-L, long noncoding RNAs (orange boxes). (B) 3′ untranslated region (UTR) and 5′ UTR in COI and COII mRNAs, respectively. Translation initiation codons (GUG and AUG) and termination codons (UAA) are indicated by green and red circles, respectively. The 3′ UTR of COI mRNA contains a mirror tRNA^{Ser} motif, and the 5′ UTR of COII mRNA contains a GAAA tetra-loop hairpin motif.
The annotated mtSSU rRNA and mtLSU rRNA genes in Atlantic cod are 952 and 1664 bp, respectively [7]. The corresponding rRNAs are highly conserved within the species [18] and well conserved between different fish species [7, 51]. The 5′ and 3′ ends of Atlantic cod mitochondrial rRNAs have been precisely mapped using different approaches. Primer extension and pyrosequencing confirmed the 5′ ends to correspond to the annotated features based on comparative sequence alignments [47, 51]. The 3′ ends were mapped by pyrosequencing and by RNA ligation sequencing [51]. Interestingly, non-template adenosines were added at both rRNAs. Whereas the 3′ end of mtSSU rRNA was found to be homogenous and mono-adenylated, the corresponding end of mtLSU rRNA was heterogeneous and oligo-adenylated [51]. The observed mtLSU rRNA heterogeneity is consistent with the notion that mitochondrial rRNAs are transcribed and processed from two different precursor RNAs, the HSP1 and HSP2 primary transcripts (Figure 3A).

5. Mitochondrial noncoding RNAs

In addition to the canonical mitochondrial genes and the newly proposed MDPs, vertebrate mitogenomes encode several noncoding RNAs [36]. The first discovered mitochondrial long noncoding RNA (lncRNA) was the human L-strand-specific 7S RNA (lncCR-L) [52, 53]. At least eight vertebrate mitochondrial lncRNAs have now been proposed and characterized [54]. Two lncRNAs correspond to the H-strand and L-strand of CR (lncCR-H and lncCR-L) [10, 18, 47, 52, 55, 56], one is an antisense chimer to partial regions of the CytB and COI mRNAs (LIPCAR) [57–59], three are mRNA antisense RNAs (lncND5, lncND6, and lncCytB) [60], and two are chimeric RNAs that involve sense and antisense mtLSUrRNAs (SncmtRNA and ASncmtRNA) [61–63]. So far, LIPCAR, rRNA chimers, and lncCR-H have been associated with human diseases [56, 57, 61, 63–66]. There are apparently a large number of small non-coding RNAs (mitosRNAs) generated from vertebrate mitochondrial transcripts [36, 67–69]. None of these mitosRNAs have been assigned to a specific function funded on experimental evidence. However, in a recent study by Riggs and Podrabsky [70], mitosRNAs were associated to a hypoxia stress response in killifish embryos.

5.1. Atlantic cod mitochondrial long noncoding RNAs

Two lncRNAs (lncCR-H and lncCR-L) have been identified and investigated in Atlantic cod mitochondria (Figure 4) [10, 18, 47]. Both lncRNAs were found to be polyadenylated but transcribed from opposite strands within the CR [10]. We showed that the Atlantic cod lncCR-L has a mutation rate and an expression level corresponding to that of Complex I mRNAs [10, 18, 47]. The lncCR-L apparently corresponds to the 7S RNA in human mitochondria [52], and recently we showed that lncCR-L is differentially expressed in a human cancer-matched cell line pair [56]. The lncCR-H was found to be highly variable in sequence and structure, both between and within Atlantic cod specimens [10, 18]. A schematic overview of the lncCR-H RNA is presented in Figure 4. Here, the noncoding T–P spacer is present at the 5′ end and includes two potential RNA hairpin structures. The T–P spacer domain is followed by a mirror tRNAPro, before entering the HTR array motifs. The HTR copy numbers vary between 2 (80 bp)
and more than 8 (>320 bp) [5, 14, 15, 18], rendering IncCR-H highly variable in size. Finally, IncCR-H terminates in a short polyA tail at TAS. Thus, IncCR-H has apparently no fixed length in Atlantic cod mitochondria and varies in size between approximately 300 and 500 nt. Interestingly, the TAS motif consists of a perfect palindromic sequence motif (TTTATAACATATGTATAAA). We found IncCR-L to terminate with a polyA tail at the same site as IncCR-H but on the opposite strand [10].

5.2. Atlantic cod mitochondrial small RNAs

The Atlantic cod mitogenomes express a number of small RNAs, revealed by SOLiD small RNA sequencing experiments (our unpublished results). Here, the majority of mitosRNA was identified as mitochondrial tRNA-derived fragments (tRFs; see [69, 70]). Interestingly, most Atlantic cod mitochondrial tRFs correspond to H-strand tRNAs, and some tRFs were differentially expressed during early developmental stages (our unpublished results). Many of the same tRF species detected in Atlantic cod have recently been noted in rainbow trout egg cells [69] and in killifish embryos [70], suggesting a conserved feature at least among some bony fishes.

The SOLiD experiments also detected several abundant small RNAs mapping to the mitochondrial CR [17]. We found three small RNA candidates generated from IncCR-L, suggesting this IncRNA to be a precursor for mitosRNA (Figure 4). Similarly, two mitosRNA were generated from IncCR-H, one corresponded to a pyrimidine-rich motif and the other to tRF-1 derived from tRNA

![Figure 4. Schematic view of CR and corresponding noncoding RNAs in Atlantic cod. tRNA genes (tRNA_{Thr}, tRNA_{Pro}, tRNA_{Phe}), T–P spacer, HTR (heteroplasmic tandem repeat array), TAS (termination associated sequence), and CSB2 (conserved sequence box 2) are indicated. The H-strand-specific IncCR-H is located at the 5′ domain of CR and is the precursor of two enriched small RNAs (above CR map). The L-strand-specific IncCR-L is located at the central domain of CR and is the precursor of three enriched small RNAs (below CR map).](http://dx.doi.org/10.5772/intechopen.75883)
mitochondria are not currently known, but we speculate that regulatory roles related to transcription elongation, mtDNA replication, or ribosome functions are likely.

6. Concluding remarks

The mitochondrial gene content and organization are highly conserved between Atlantic cod and human and strongly support a common functional platform. Similarly, the mitochondrial transcripts generating canonical mRNAs and structural RNAs are surprisingly similar. What about the newly proposed MDPs and noncoding RNAs? Are there any lineage-specific differences? Research is still in its infancy, but recent findings suggest conservations between fish and mammals. More experimental studies in Atlantic cod and model systems like zebrafish are highly encouraged, including investigations of the fascinating mitochondrial swinger RNAs [24, 71, 72]. Mitochondrial-derived noncoding RNAs need to be profiled and further investigated in adult tissue types during normal and stress conditions, as well as at various developmental stages. A first step could be to study the intracellular location by in situ RNA hybridization and then ask if the noncoding RNAs are confined to the mitochondrial compartment or exported to the cytoplasm or other cellular compartments. Our studies in Atlantic cod indicate that at least two of the mitochondrial lncRNAs may serve as precursors for small RNAs. We conclude that vertebrate mitogenomes encode a significant number of gene products in addition to the 37 canonical OxPhos proteins, rRNAs, and tRNAs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] Boore JL. Animal mitochondrial genomes. Nucleic Acids Research. 1999;27:1767-1780. DOI: 10.1093/nar/27.8.1767

[2] NCBI Public Database. 2018. Available from: https://www.ncbi.nlm.nih.gov/genome/organelle/ [Accessed: February 10, 2018]

[3] Bakke I, Johansen SD. Molecular phylogenetics of Gadidae and related Gadiformes based on mitochondrial DNA sequences. Marine Biotechnology. 2005;7:61-69. DOI: 10.1007/s10126-004-3131-0

[4] Johansen SD, Coucheeron DH, Andreassen M, Karlsen BO, Furmanek T, Jørgensen TE, Emblem Å, Breines R, Nordeide JT, Moom T, Nederbragt AJ, Stenseth NC, Jakobsen KS. Large-scale sequence analyses of Atlantic cod. New Biotechnology. 2009;25:263-271. DOI: 10.1016/j.nbt.2009.03.014

[5] Johansen S, Guddal PH, Johansen T. Organization of the mitochondrial genome of Atlantic cod. Nucleic Acids Research. 1990;18:411-419. DOI: 10.1093/nar/18.3.411

[6] Johansen S, Johansen T. Sequence analysis of 12 structural genes and a novel non-coding region from mitochondrial DNA of Atlantic cod. Biochimica et Biophysica Acta. 1994;1218:213-217. DOI: 10.1016/0167-4781(94)90015-9

[7] Johansen S, Bakke I. The complete mitochondrial DNA sequence of Atlantic cod (Gadus morhua): Relevance to taxonomic studies among codfishes. Molecular Marine Biology and Biotechnology. 1996;5:203-214

[8] Kühnlbrandt W. Structure and function of mitochondrial membrane protein complexes. BMC Biology. 2015;13:89. DOI: 10.1186/s12915-015-0201-x

[9] Johansen S, Johansen T. The putative origin of heavy strand replication (oriH) in mitochondrial DNA is highly conserved among teleost fishes. DNA Sequence – Journal of DNA Sequencing and Mapping. 1993;3:397-399. DOI: 10.3109/10425179309020843

[10] Jørgensen TE, Bakke I, Ursvik A, Andreassen M, Moom T, Johansen SD. An evolutionary preserved intergenic spacer in gadiform mitogenomes generates a long noncoding RNA. BMC Evolutionary Biology. 2014;14:182. DOI: 10.1186/s12862-014-0182-3

[11] Wanrooij S, Miralles Fusté J, Stewart JB, Wanrooij PH, Samuelsson T, Larsson N-G, Gustafsson CM, Falkenberg M. In vivo mutagenesis reveals that OriL is essential for mitochondrial DNA replication. EMBO Report. 2012;13:1130-1137. DOI: 10.1038/embor.2012.161

[12] Bailey LJ, Doherty AJ. Mitochondrial DNA replication: A PrimPol perspective. Biochemical Society Transactions. 2017;45:513-529. DOI: 10.1042/BST20160162

[13] Bakke I, Shields GF, Johansen S. Sequence characterization of a unique intergenic spacer in gadiformes mitochondrial DNA. Marine Biotechnology. 1999;1:411-415. DOI: 10.1007/PL00011797
[14] Arnason E, Rand DM. Heteroplasmy of short tandem repeats in mitochondrial DNA of Atlantic cod. Genetics. 1992;132:211-220. DOI: 10.3109/19401736.2010.551659

[15] Kijewska A, Burzynski A, Wenne R. Variation in the copy number of tandem repeats of mitochondrial DNA in the North-East Atlantic cod populations. Marine Biology Research. 2009;5:186-192. DOI: 10.1080/17451000802277408

[16] Karlsen BO, Emblem Å, Jørgensen TE, Klingan KA, Nordeide JT, Moum T, Johansen SD. Mitogenome sequence variation in migratory and stationary ecotypes of North-East Atlantic cod. Marine Genomics. 2014;15:103-108. DOI: 10.1016/j.margen.2014.01.001

[17] Karlsen BO, Klingan K, Emblem Å, Jørgensen TE, Jueterbock A, Furmanek T, Hoarau G, Johansen SD, Nordeide JT, Moum T. Genomic divergence between the migratory and stationary ecotypes of Atlantic cod. Molecular Ecology. 2013;22:5098-5111. DOI: 10.1111/mec.12454

[18] Jørgensen TE. Molecular and evolutionary characterization of the Atlantic cod mitochondrial genome [PhD thesis]. Norway: Nord University; 2018

[19] Carr SM, Marshall HD. Intraspecific phylogeographic genomics from multiple complete mtDNA genomes in Atlantic cod (Gadus morhua): Origins of the ‘codmother,’ transatlantic vicariance and midglacial population expansion. Genetics. 2008;180:381-389. DOI: 10.1534/genetics.108.089730

[20] Ingman M, Gyllensten U. Analysis of the complete human mtDNA genome: Methodology and inferences for human evolution. The Journal of Heredity. 2001;92:454-461. DOI: 10.1093/jhered/92.6.454

[21] Ingman M, Gyllensten U. mtDB: Human mitochondrial genome database, a resource for population genetics and medical sciences. Nucleic Acids Research. 2006;34:D749-D751. DOI: 10.1093/nar/gkj010

[22] Diroma MA, Calabrese C, Simone D, Santorsola M, Calabrese FM, Gasparre G, Attimonelli M. Extraction and annotation of human mitochondrial genomes from 1000 genomes whole exome sequencing data. BMC Genomics. 2014;15(Supp l3):S2. DOI: 10.1186/1471-2164-15-S3-S2

[23] Flynn T, Signal B, Johnson SL, Gemmell NJ. Mitochondrial genome diversity among six laboratory zebrafish (Danio rerio) strains. Mitochondrial DNA Part A. 2016;27:4364-4371. DOI: 10.3109/19401736.2015.1089536

[24] Seligmann H. Coding constraints modulate chemically spontaneous mutational replication gradients in mitochondrial genomes. Current Genomics. 2012;13:37-43. DOI: 10.2174/138920212799034802

[25] Seligmann H. Chap. 6: Mutation patterns due to converging mitochondrial replication and transcription increase lifespan, and cause growth rate-longevity tradeoffs. In: Seligmann H, editor. DNA Replication – Current Advances. Rijeka: InTech; 2011. pp. 137-166. DOI: 10.5772/791
[26] Kim S-J, Xiao J, Wan J, Cohen P, Yen K. Mitochondrially derived peptides as novel regulators of metabolism. The Journal of Physiology. 2017;595:6613-6621. DOI: 10.1113/JP274472

[27] Cobb LJ, Lee C, Xiao J, Yen K, Wong RG, Nakamura HK, Mehta HH, Gao Q, Ashur C, Huffman DM, Wan J, Muzumdar R, Barzilai N, Cohen P. Naturally occurring mitochondrial-derived peptides are age-dependent regulators of apoptosis, insulin sensitivity, and inflammatory markers. Aging. 2016;8:796-809. DOI: 10.18632/aging.100943

[28] Seligmann H. Chimeric mitochondrial peptides from contiguous regular and swinger RNA. Computational and Structural Biotechnology Journal. 2016;14:283-297. DOI: 10.1016/j.csbj.2016.06.005

[29] Lee C, Yen K, Cohen P. Humanin: A harbinger of mitochondrial-derived peptides? Trends in Endocrinology and Metabolism. 2013;24:222-228. DOI: 10.1016/j.tem.2013.01.005

[30] Lee C, Zeng J, Drew BG, Sallam T, Martin-Montalvo A, Wan J, Kim S-J, Mehta H, Hevener AL, de Cabo R, Cohen P. The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance. Cell Metabolism. 2015;21:443-454. DOI: 10.1016/j.cmet.2015.02.009

[31] Hashimoto Y, Niikura T, Tajima H, Yasukawa T, Sudo H, Ito Y, Kita Y, Kawasaki M, Kouyama K, Doyu M, Sobue G, Koide T, Tsuji S, Lang J, Kurokawa K, Nishimoto I. A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer’s disease genes and Aβ. Proceedings of the National Academy of Sciences of the United States of America. 2001;98:6336-6341. DOI: 10.1073/pnas.101133498

[32] Guo B, Zhai D, Cabezas E, Welsh K, Nouraini S, Satterthwait AC, Reed JC. Humanin peptide suppresses apoptosis by interfering with Bax activation. Nature. 2003;423:456-461. DOI: 10.1038/nature01627

[33] Paharkova V, Alvarez G, Nakamura H, Cohen P, Lee K-W. Rat Humanin is encoded and translated in mitochondria and is localized to the mitochondrial compartment where it regulates ROS production. Molecular and Cellular Endocrinology. 2015;413:96-100. DOI: 10.1016/j.mce.2015.06.015

[34] Capt C, Passamonti M, Breton S. The human mitochondrial genome may code for more than 13 proteins. Mitochondrial DNA Part A. 2016;27:3098-3101. DOI: 10.3109/19401736.2014.1003924

[35] Ming W, Lu G, Xin S, Huanyu L, Yinghao J, Xiaoying L, Chengming X, Banjun R, Li W, Zifan L. Mitochondria related peptide MOTS-c suppresses ovariectomy-induced bone loss via AMPK activation. Biochemical and Biophysical Research Communications. 2016;476:412-419. DOI: 10.1016/j.bbrc.2016.05.135

[36] Mercer TR, Neph S, Dinger ME, Crawford J, Smith MA, Shearwood A-MJ, Haugen E, Bracken CP, Rackham O, Stamatoyannopoulos JA, Filipovska A, Mattick JS. The human mitochondrial transcriptome. Cell. 2011;146:645-658. DOI: 10.1016/j.cell.2011.06.051
[37] Gustafsson CM, Falkenberg M, Larsson N-G. Maintenance and expression of mammalian mitochondrial DNA. Annual Review of Biochemistry. 2016;85:133-160. DOI: 10.1146/annurev-biochem-060815-014402

[38] Fernandez-Silva P, Acin-Perez R, Fernandez-Vizarra E, Perez-Martos A, Enriques JA. In vivo and in organelle analyses of mitochondrial translation. Methods in Cell Biology. 2007;80:571-588. DOI: 10.1016/S0091-679X(06)80028-2

[39] Falkenberg M, Larsson N-G, Gustafsson CM. DNA replication and transcription in mammalian mitochondria. Annual Review of Biochemistry. 2007;76:679-699. DOI: 10.1146/annurev.biochem.76.060305.152028

[40] Temperley RJ, Wydro M, Lightowlers RN, Chrzanowska-Lightowlers ZM. Human mitochondrial mRNAs-like members of all families, similar but different. Biochimica et Biophysica Acta. 1797;2010:1081-1085. DOI: 10.1016/j.bbabio.2010.02.036

[41] Shutt TE, Lodeiro MF, Cotney J, Cameron CE, Shadel GS. Core human mitochondrial transcription apparatus is a regulated two-component system in vitro. Proceedings of the National Academy of Sciences of the United States of America. 2010;107:12133-12138. DOI: 10.1073/pnas.0910581107

[42] Suzuki T, Nagao A, Suzuki T. Human mitochondrial tRNAs: Biogenesis, function, structural aspects, and diseases. Annual Review of Genetics. 2011;45:299-329. DOI: 10.1146/annurev-genet-110410-132531

[43] Brown A, Amunts A, Bai XC, Sugimoto Y, Edwards PC, Murshudov G, Scheres SHW, Ramakrishnan V. Structure of the large ribosomal subunit from human mitochondria. Science. 2014;346:718-722. DOI: 10.1126/science.1258026

[44] Ott M, Amunts A, Brown A. Organization and regulation of mitochondrial protein synthesis. Annual Review of Biochemistry. 2016;85:77-101. DOI: 10.1146/annurev-biochem-060815-014334

[45] Rorbach J, Miniczuk M. The post-transcriptional life of mammalian mitochondrial RNA. Biochemical Journal. 2012;444:357-373. DOI: 10.1042/BJ20112208

[46] Asin-Cayuela J, Gustafsson CM. Mitochondrial transcription and its regulation in mammalian cells. Trends in Biochemical Sciences. 2007;32:111-117. DOI: 10.1016/j.tibs.2007.01.003

[47] Coucheron DH, Nymark M, Breines R, Karlsen BO, Andreassen M, Jørgensen TE, Moum T, Johansen SD. Characterization of mitochondrial mRNAs in codfish reveals unique features compared to mammals. Current Genetics. 2011;57:213-222. DOI: 10.1007/s00294-011-0338-2

[48] Abramovitz DL, Pyle AM. Remarkable morphological variability of a common RNA folding motif: The GNRA tetraloop-receptor interaction. Journal of Molecular Biology. 1997;266:493-506

[49] Slomovic S, Laufer D, Geiger D, Schuster G. Polyadenylation and degradation of human mitochondrial RNA: The prokaryotic past leaves its mark. Molecular and Cellular Biology. 2005;25:6427-6435. DOI: 10.1128/MCB.25.15.6427-6435.2005
Hällberg BM, Larsson N-G. Making proteins in the powerhouse. Cell Metabolism. 2014;20:226-240. DOI: 10.1016/j.cmet.2014.07.001

Bakke I, Johansen S. Characterization of mitochondrial ribosomal RNA genes in gadi-formes: Sequence variation, secondary structure features, and phylogenetic implications. Molecular Phylogenetics and Evolution. 2002;25:87-100. DOI: 10.1016/S1055-7903(02)00220-8

Ojala D, Crews S, Montoya J, Gelfand R, Attardi G. A small polyadenylated RNA (7S RNA), containing a putative ribosome attachment site, maps near the origin of human mitochondrial DNA replication. Journal of Molecular Biology. 1981;150:303-314. DOI: 10.1016/0022-2836(81)90454-X

Chang DD, Clayton DA. Precise identification of individual promoters for transcription of each strand of human mitochondrial DNA. Cell. 1984;36:635-643. DOI: 10.1016/0092-8674(84)90343-X

Dietrich A, Wallet C, Iqbal RK, Gualberto JM, Lotfi F. Organellar non-coding RNAs: Emerging regulation mechanisms. Biochimie. 2015;117:48-62. DOI: 10.1016/j.biochi.2015.06.027

Gao S, Tian X, Chang H, Sun Y, Wu Z, Cheng Z, Dong P, Zhao Q, Ruan J, Bu W. Two novel IncRNAs discovered in human mitochondrial DNA using PacBio full-length transcriptome data. Mitochondrion. 2018;38:41-47. DOI: 10.1016/j.mito.2017.08.002

Hedberg A, Knutsen E, Lövhaugen AS, Jørgensen TE, Perander M, Johansen SD. Cancer-specific SNPs originate from low-level heteroplasmic variants in human mitochondrial genomes of a matched cell line pair. Mitochondrial DNA Part A. 2018;29. DOI: 10.1080/24701394.2018.1461852

Kumarswamy R, Bauters C, Volkmann I, Maury F, Fetisch J, Holzmann A, Lemesle G, de Groote P, Pinet F, Thum T. Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. Circulation Research. 2014;114:1569-1575. DOI: 10.1161/CIRCRESAHA.114.303915

de Gonzalo-Calvo D, Kenneweg F, Bang C, Toro R, van der Meer RW, Rijzewijk LJ, Smit JW, Lamb HJ, Llorente-Cortes V, Thum T. Circulating long-noncoding RNAs as biomarkers of left ventricular diastolic function and remodelling in patients with well-controlled type 2 diabetes. Scientific Reports. 2016;6:37354. DOI: 10.1038/srep37354

Zhang Z, Gao W, Long QQ, Zhang J, Li YF, Liu DC, Yan JJ, Yang ZJ, Wang LS. Increased plasma levels of IncRNA H19 and LIPCAR are associated with increased risk of coronary artery disease in a Chinese population. Scientific Reports. 2017;7:7491. DOI: 10.1038/s41598-017-07611-z

Rackham O, Shearwood A-MJ, Mercer TR, Davies SMK, Mattick JS, Filipovska A. Long noncoding RNAs are generated from the mitochondrial genome and regulated by nuclear-encoded proteins. Ribonucleic Acid. 2011;17:2085-2093. DOI: 10.1261/rna.029405.111
[61] Burzio VA, Villota C, Villegas J, Landerer E, Boccardo E, Villa LL, Martinez R, Lopez C, Gaete F, Toro V, Rodriguez X, Burzio LO. Expression of a family of noncoding mitochondrial RNAs distinguishes normal from cancer cells. Proceedings of the National Academy of Sciences of the United States of America. 2009;106:9430-9434. DOI: 10.1073/pnas.0903086106

[62] Landerer E, Villegas J, Burzio VA, Oliveira L, Villota C, Lopez C, Restovic F, Martinez R, Castillo O, Burzio LO. Nuclear localization of the mitochondrial ncRNAs in normal and cancer cells. Cellular Oncology. 2011;34:297-305. DOI: 10.1007/s13402-011-0018-8

[63] Vidaurre S, Fitzpatrick C, Burzio VA, Briones M, Villita C, Villegas J, Echenique J, Oliveira-Cruz L, Araya M, Borgna V, Socias T, Lopez C, Avila R, Burzio LO. Down-regulation of the antisense mitochondrial non-coding RNAs (ncRNAs) is a unique vulnerability of cancer cells and a potential target for cancer therapy. The Journal of Biological Chemistry. 2014;289:27182-27198. DOI: 10.1074/jbc.M114.558841

[64] Rivas A, Burzio V, Landerer E, Borgna V, Gatica S, Avila R, Lopes C, Villota C, de la Fuente R, Echenique J, Burzio LO, Villegas J. Determination of the differential expression of mitochondrial long non-coding RNAs as a noninvasive diagnosis of bladder cancer. BMC Urology. 2012;12:37. DOI: 10.1186/1471-2490-12-37

[65] Villota C, Campos A, Vidaurre S, Oliveira-Cruz L, Boccardo E, Burzio VA, Varas M, Villegas J, Villa LL, Valenzuela PDT, Socias M, Roberts S, Burzio LO. Expression of mitochondrial non-coding RNAs (ncRNAs) is modulated by high risk human papillomavirus (HPV) oncogenes. The Journal of Biological Chemistry. 2012;287:21303-21315. DOI: 10.1074/jbc.M111.326694

[66] Varas-Godoy M, Lladser A, Farfan N, Villota C, Villegas J, Tapia JC, Burzio LO, Burzio VA, Valenzuela PDT. In vivo knockdown of antisense non-coding mitochondrial RNAs by a lentiviral-encoded shRNA inhibits melanoma tumor growth and lung colonization. Pigment Cell and Melanoma Research. 2018;31:64-72. DOI: 10.1111/pcmr.12615

[67] Sripada L, Tomar D, Prajapati P, Singh R, Singh AK, Singh R. Systematic analysis of small RNAs associated with human mitochondria by deep sequencing: Detailed analysis of mitochondrial associated miRNA. PLoS One. 2012;7:e44873. DOI: 10.1371/journal.pone.0044873

[68] Ro S, Ma HY, Park C, Ortogero N, Song R, Hennig GW, Zheng H, Lin YM, Moro L, Hsieh JT, Yan W. The mitochondrial genome encodes abundant small noncoding RNAs. Cell Research. 2013;23:759-774. DOI: 10.1038/cr.2013.37

[69] Ma H, Weber GM, Wei H, Yao J. Identification of mitochondrial genome-encoded small RNAs related to egg deterioration caused by postovulatory aging in rainbow trout. Marine Biotechnology. 2016;18:584-597. DOI: 10.1007/s10126-016-9719-3

[70] Riggs CL, Podrabsky JE. Small noncoding RNA expression during extreme anoxia tolerance of annual killifish (Austrofundulus limnaeus) embryos. Physiological Genomics. 2017;49:505-518. DOI: 10.1152/physiolgenomics.00016.2017
[71] Seligmann H. Sharp switches between regular and swinger mitochondrial replication: 16S rDNA systematically exchanging nucleotides $A \leftrightarrow T + C \leftrightarrow G$ in the mitogenome of *Kamimuria wangii*. Mitochondrial DNA Part A. 2016;27:2440-2446. DOI: 10.3109/19401736.2015.1033691

[72] Seligmann H. Reviewing evidence for systematic deletions, nucleotide exchange, and expanded codons, and peptide clusters in human mitochondria. Bio Systems. 2017;160:10-24. DOI: 10.1016/j.biosystems.2017.08.002
