Mitochondrial DNA

http://informahealthcare.com/mdn
ISSN: 1940-1736 (print), 1940-1744 (electronic)

Mitochondrial DNA, Early Online: 1–4
 DOI: 10.3109/19401736.2014.1003924

informa
healthcare

SHORT COMMUNICATION

The human mitochondrial genome may code for more than 13 proteins

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Abstract

The human mitochondrial (mt) DNA is commonly described as a small, maternally inherited molecule that encodes 13 protein components of the oxidative phosphorylation system and 24 structural RNAs required for their translation. However, recent studies indicate that the human mtDNA has a larger functional repertoire than previously believed. This paper briefly summarizes these studies, which suggest to reconsider our way to describe the human mitochondrial DNA as it may code for more than 13 proteins.

Keywords

Genomics, humanin, mitochondrial DNA, mtDNA, open reading frames

History

Received 12 November 2014
Revised 3 December 2014
Accepted 5 December 2014
Published online 29 January 2015

Introduction

Past and current scientific literature is basically unanimous in describing the animal mitochondrial (mt) genome as a small, maternally inherited DNA that encodes only 37 genes, 13 of which are key proteins of the oxidative phosphorylation (OXPHOS) system, whereas the remaining 24 genes are required for their translation (Scheffler, 2008). The basic set of mitochondrial protein-coding genes originally found in humans and later reported in most vertebrate and invertebrate species, consists, with few exceptions, of *cob* (apocytochrome b), *cox1*,*2*,*3* (cytochrome *c* oxidase subunits), *atp6*,*8* (ATPase subunits), and *nad1*,*2*,*3*,*4*,*4L*,*5*,*6* (NADH dehydrogenase subunits) (Anderson et al., 1981; Breton et al., 2014; Gissi et al., 2008). This limited genetic scope (i.e. genome size and gene content available for novel functions) of animal mitochondrial DNA has thus traditionally constrained considerations of its potential functional roles in an organism, including in humans. However, recent studies indicate that the human mtDNA has a larger functional repertoire than previously thought (Breton et al., 2014; Faure et al., 2011; Lee et al., 2013). We feel that it is important and necessary to briefly discuss these studies and we suggest reconsidering our way to describe the human mitochondrial DNA as it may code for more than 13 proteins.

The *humanin* gene

Humanin (HN) is a 24-amino acid peptide that has been discovered more than a decade ago and initially identified as a neuroprotective factor against Alzheimer's disease-related neurotoxicity (Hashimoto et al., 2001). There is now evidence that HN possesses both intra- and extra-cellular modes of action: at the intracellular level, HN is involved in the mitochondrial-nuclear retrograde signaling and it interacts with pro-apoptotic proteins to prevent apoptosis, whereas at the extracellular level, HN regulates important cellular processes such as survival, metabolism, and inflammation (Lee et al., 2013). Since its discovery, there has been controversy about whether humanin is transcribed from genomic or mitochondrial DNA. The originally identified 1567-base cDNA containing the small HN open reading frame (ORF) was 99.9% identical with a fragment of the mitochondrial 16S rRNA gene (Hashimoto et al., 2001; Maximov et al., 2002) (Figure 1). If transcribed from the mitochondrial genome, one first scenario is that HN is produced within mitochondria and is exported, for example, through a mitochondrial pathway of peptide efflux as described in Young et al. (2001). A second scenario is that 16S rRNA is first translocated to cytoplasm, by a still unknown mechanism, and is translated into HN protein. Even if the translational site remains undetermined, HN has been shown to be biologically effective when synthesized using both mitochondrial and cytoplasmic codes (Guo et al., 2003). Alternatively, the humanin gene could have been transferred to the nuclear genome, a phenomenon known as NUMT (nuclear mitochondrial DNA), which is supported, in this case, by the presence of several nuclear regions with >90% similar to the original HN cDNA, each of which includes an intact ORF for a HN-like peptide (Maximov et al., 2002; Nishimoto et al., 2004). Gene expression data showed that some of these regions might encode functional peptides with antiapoptotic properties, but none of the peptide sequences deduced from NUMTs are fully identical to the original cDNA clones isolated from the brain of a patient with Alzheimer's disease or from HeLa cells, which were 99 and 100% identical to the mtDNA-encoded 16S rRNA gene (Bodzioch et al., 2009; Hashimoto et al., 2001; Lee et al., 2013). We performed a DNA BLAST analysis (Ensembl database) of the original humanin sequence (AY029066.1; Hashimoto et al., 2001)

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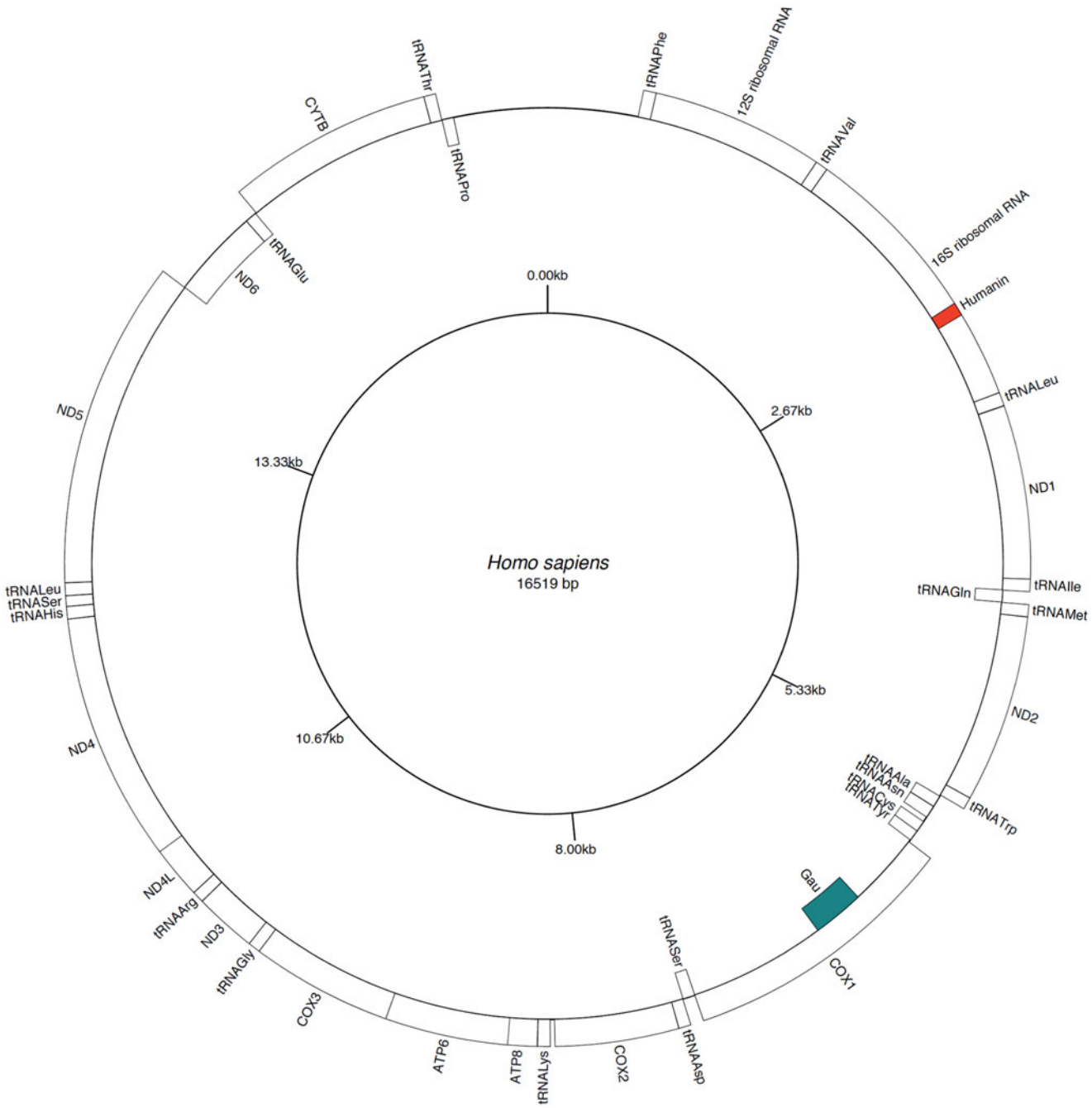

Figure 1. Gene map of the human mitochondrial genome. In white, the set of 37 genes (13 protein-coding genes, 22 tRNAs, and two rRNAs) typical of most metazoan mitochondrial genomes. Genes positioned inside the circle are encoded on the light strand whereas genes outside are encoded on the heavy strand. The protein-coding gene humanin found in the 16S rRNA gene (in red). The protein-coding gene *GAU* located on the complementary strand of *cox1* (in green).

against the human genome and found 16 hits with >89% similar to the humanin sequence. Specifically, 10 nuclear regions with 89–94% similarity and five nuclear regions with 97.3–98.7% similar to the original HN cDNA have been identified. Six out of these 15 nuclear regions included an intact ORF for a HN-like peptide (Figure 2). Only one hit was 100% similar to the humanin sequence, i.e. the region from 2633 to 2707 bp in the 16S rRNA gene of the human mitochondrial genome. Thus, the existence of NUMT-derived humanin has not been ruled out yet, actually, there are most likely many nuclearly encoded HN-like isoforms (e.g. Mottaghi-Dastjerdi et al., 2014). However, in addition to the above-mentioned DNA evidence, strong circumstantial data indicate that the mitochondrial 16S rRNA gene encodes at least one antiapoptotic humanin peptide (Harada et al., 2004; Lee et al., 2013; Maximov et al., 2002). First, the widely observed up-regulation of the 16S rRNA transcription in cancer cells has been proposed to enhance humanin expression interfering with apoptosis and thus sustaining cancer development (Maximov et al., 2002). Furthermore, northern blot analysis using total RNA from HeLa cells and the humanin ORF as the probe showed hybridization to a fragment that was identical in size to mitochondrial 16S rRNA, whereas total RNA from $\rho 0$ HeLa cells, which lack mtDNA, did not show any significant hybridization (Lee et al., 2013). Lastly, it has been shown that formylated humanin has enhanced activity compared with ''regular'' humanin, and N-termini formylation is a hallmark of mitochondrion-encoded proteins (Harada et al., 2004). For all these reasons, humanin is highly likely to be encoded within the mtDNA.

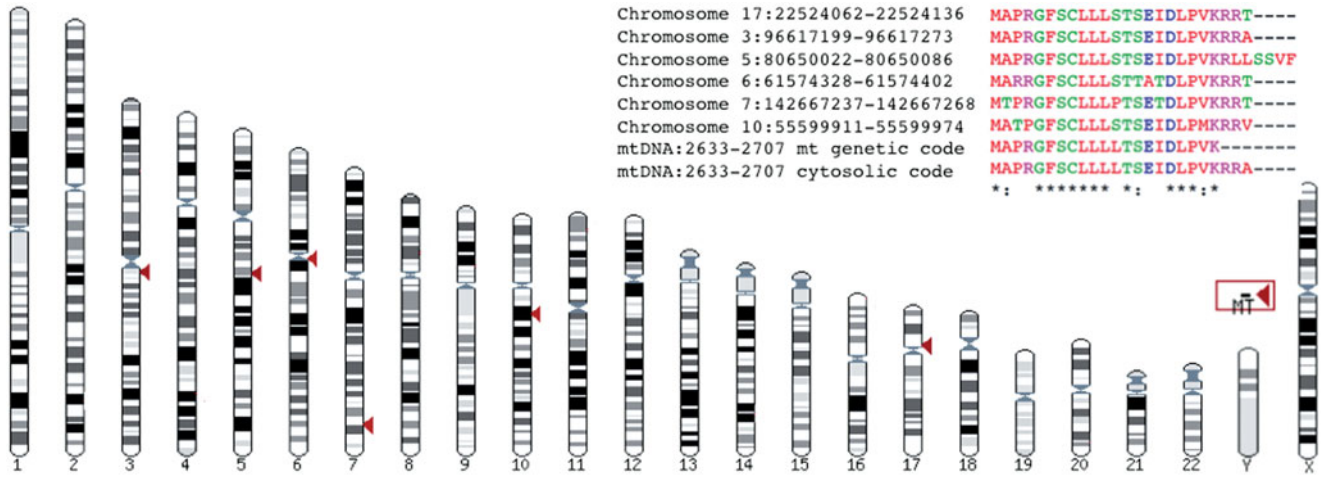

Figure 2. Distribution of HN BLAST hits in the human chromosomes and the mitochondrial (mt) genome. Arrows indicate BLAST hits that included an intact ORF for a HN-like peptide. An alignment of the deduced protein sequences using T COFFEE is shown.

The *gau* gene

Another mtDNA-encoded protein gene has recently been identified on the complementary strand of *cox1* in humans, but also in other metazoans, protists, plants, fungi, and alpha-proteobacteria (Faure et al., 2011) (Figure 1). In addition to its ubiquitous presence, the reason why the gene has been named *gau* for Gene Antisense Ubiquitous, strong arguments indicate that the *gau* ORF indeed encodes a functional protein: (i) it is evolving under purifying selection, (ii) the deduced *GAU* proteins share some conserved amino acid signatures and structure among different taxa, suggesting a possible conserved function, (iii) *gau* has been identified in sense-oriented ESTs with poly(A) tails, (iv) immunohistochemical experiments using an anti-*GAU* monoclonal antibody showed a mitochondrial-specific signal in human cells, and (v) BLAST analyses suggested that no part of any known human proteins exhibits a high level of amino acid identity with the peptide antigen that was used for immunization and antibody production (Faure et al., 2011). As for humanin, potentially functional and similar but not identical *gau* regions have been found in the nuclear genome. However, none of the deduced protein sequence possesses a mitochondrial signal peptide (Faure et al., 2011), a result that is not in line with the intramitochondrial localization of *GAU* observed using the anti-*GAU* antibody. According to the authors, the most parsimonious hypothesis is that *gau* is a mtDNA-encoded protein gene, providing evidence for antisense overlapping functional open reading frames in mitochondrial genomes.

Conclusion

The data presented above for humanin and *gau* suggest that it is highly probable that several previously undetected protein-coding genes may occur in the human mitochondrial genome, and also in other organisms (Breton et al., 2014; Faure et al., 2011; Lee et al., 2013). In humans, the presence of additional ORFs with potential functionality has already been confirmed by bioinformatics analyses in the 16s rRNA gene, along with humanin, but also in the 12S rRNA gene and in the D-loop (Seligmann, 2013a). Some of them might be translated to peptides playing a role as significant as that of Humanin. Notably, there is compelling evidence for small nuclear open reading frames (sORF) encoding biologically active peptides of 11–32 amino acids in length (Andrew & Rothnagel, 2014; Kondo et al., 2010). Similarly, sORF could be identified in mitochondrial intergenic regions defined as ''noncoding'', but as described above, even with no intergenic regions, the protein-coding capacity of the mtDNA can be increased with the transcription of protein genes within rRNA genes, protein genes from the same strand but in different reading frames or protein genes from different strands. All these cases have already been widely observed in viruses, bacteria, plasmids, and even in mitochondria (Faure et al., 2011). Other mechanisms that could enable the human mitochondrial genome to code for additional proteins without an increase in size include, for example, (i) the occurrence of antisense antitermination (suppressor) tRNAs or imported cytosolic tRNAs that could translate stop codons present in overlapping ORFs within frameshifted and antisense sequences of regular mitochondrial protein-coding genes (e.g. Faure et al., 2011; Seligman, 2013a); (ii) the reorganization of genetic information at the level of RNA or protein (e.g. Moreira et al., 2012; Seligman, 2013b,c); and (iii) the occurrence of tRNAs with expanded anticodon loops, that could translate expanded anticodons, or tetracodons (e.g. Seligman, 2012a,b). Although speculative, this last hypothesis is supported by the observation that the numbers of tetracodons coevolve with BLAST-predicted overlapping tetracoded mitochondrial ORFs (Seligman, 2012a,b), that tetracoding apparently increases with body temperature (Seligmann & Labra, 2013), suggesting that thermodynamically more stable codon–anticodon interactions (four rather than three nucleosides interact) improve translation at high temperatures, and that tetracoding has been suggested as the ancestral vertebrate genetic code (Gonzalez et al., 2012).

In conclusion, for decades, the human mitochondrial genome has been depicted as having a very limited coding capacity with only 13 protein-coding genes. The studies presented here challenge these paradigms, prompting us to reconsider our way to describe it.

Declaration of interest

The authors report that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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