Int.J.Curr.Microbiol.App.Sci (2016) *5*(6): 179-189

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 6 (2016) pp. 179-189**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.506.022

Identification of New Genes Involved in mtDNA Maintenance in *Caenorhabditis elegans* that could Represent Candidate Genes for Mitochondrial Diseases

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Keywords

candidate genes, mtDNA, EtBr, orthologs, mitochondrial, diseases.

Article Info

Accepted:
12 May 2016
Available Online:
10 June 2016

A B S T R A C T

Identification of genes involved in mtDNA maintenance in *Caenorhabditis elegans* that could represent candidate genes for mitochondrial diseases associated with quantitative or qualitative mtDNA abnormalities was performed using our previous validated method of RNAi combined with ethidium bromide. This was to knock down *C. elegans* genes homologous to human genes known to be involved in mtDNA stability. Silencing of these candidate genes were repeated again without EtBr to measure the worm's mtDNA content and the efficiency of gene silencing by Q-PCR and RT-Q-PCR. Among the 182 genes encoding mitochondrial proteins tested randomly, we found that inactivation of 25 of them lead to EtBr sensitivity. Out of these genes, 20 fall into four main categories of metabolism (*GOT2, WWOX, ALDH1L2, WWOX, AGMAT* and *ACOT9*), protein synthesis (*TRMU, MRPL47, MRPS14, MRPS18C, MRPL4* and *MRPL12*), mitochondrial morphology (*OPA1,*) and respiratory chain oxidative phosphorylation (*ATP5F1, NDUFS4, FH, CYP2C8, CYP3A7, CYP2C8 and CYP2C8).* The human orthologs of these genes as presented in this study could be considered as reservoir of new genes which will represent a helpful tool and knowledge for mitochondrial diseases, as they would be considered as candidate genes for patients.

Introduction

Until recently, our knowledge on mitochondrial dysfunction was confined to the mitochondrial compartment, but it is now known that the origin of mitochondrial dysfunctions go beyond the mitochondrial compartment in humans as shown by *p53R2* mutations (Bourdon *et al.*, 2007).

Mitochondrial biogenesis is a very complex cellular process that requires the coordination of several mechanisms involving nuclear-mitochondrial communication, mitochondrial protein expression and import, mtDNA gene expression, assembly of multisubunit enzyme complexes, regulation of mitochondrial fission and fusion as well as mitochondrial turnover in response to various stimuli. Disruption of any of these processes can lead to defective mitochondrial function and therefore to a disease state. Several gene mutations have been already found in some patients or families but the disease causing gene has still to be determined in 80% of cases (Schaefera *et al*., 2004; Rötig, 2010).

In human, either qualitative or quantitative anomalies of the mitochondrial DNA (mtDNA) result in various types of mitochondrial diseases, notably serious illness especially in children. Few genes are known in human that lead to the instability of the mitochondrial genomes, most of them have been discovered from intensive researchs on patients suffering of a mitochondrial disease (Sarzi *et al*., 2007). The extensive conservation of mitochondrial structure, composition, and function across evolution offers a unique opportunity to expand the understanding of human mitochondrial biology and disease.

Most of the human genes involved in mtDNA maintenance have been identified by sequence homology with yeast genes. However, notable differences in mtDNA structure and dynamics between yeast and human did not make it a perfect tool to study the mtDNA maintenance. Indeed, the mitochondrial genome, 16.6 kb in human and 85.8 kb in yeast, is predominantly linear in yeast but is circular in human (Burger *et al*., 2003; Legros *et al*., 2004; Williamson 2002). Again, heteroplasmy is very frequently observed for mtDNA mutations in human, whereas yeast cannot normally maintain stably heteroplasmy (Shoubridge, 2001). Due to these differences, it is still believed that several other genes are yet to be characterized and that a pluricellular organism such as *C. elegans* can be helpful for this purpose.

The success of *C. elegans* as a model organism in biological research is attributed to a number of biological and easy handling properties of the worm. Addo *et al*., (2010) have shown that, suppression by RNA interference of genes involved in mtDNA replication such as polg-1, encoding the mitochondrial DNA polymerase, results in reduced mtDNA copy number but in a normal phenotype of the F1 worms. By combining RNAi of genes involved in mtDNA maintenance and EtBr exposure, Addo *et al*., (2010) were able to reveal a strong and specific phenotype (developmental larval arrest) associated to a severe decrease of mtDNA copy number.

It is therefore important to identify new genes involved in mtDNA maintenance in *C. elegans* that could represent candidate genes for mitochondrial diseases associated with quantitative or qualitative mtDNA abnormalities. Genomic-scale approaches have proposed a human mitochondrial proteome as MitoP2 (Andreoli *et al*., 2004). Ryohei *et al.* (2008) also used the information available for human mitochondrial proteins in the MitoP2 to search for *C. elegans* genes encoding mitochondrial proteins. In total, 1009 putative genes were identified by a BLAST search using 719 human proteins (Ryohei *et al*., 2008).

This study therefore aims by investigating in *C. elegans* the genes encoding mitochondrial

proteins by undertaken a large scale screening of *C. elegans* genes. We began to perform this study by inactivation of genes encoding mitochondrial proteins by knocking down through RNAi *C. elegans* genes encoding potential mitochondrial proteins.

Material and Methods

Media and cultivation of *E. coli*

Luria-Bertani-Media (LB) (Sambrook *et al.*, 1989): 1% (w/v) Tryptone; 0,5% (w/v) yeast extract; 1% (w/v) NaCl pH 7,5. For solid media 1,5% (w/v), Agar was added before sterilization. For plasmid selection, the antibiotic Ampicillin was used at a concentration of 0,1 mg/ml. *E. coli* was cultivated in Luria-Bertani-Medium over night at 37°C. Liquid cultures were incubated with shaking (150 rpm). To select transformants the antibiotic Ampicillin (100 mg/L) was added to the media.

Strain and Growth Conditions

The *C. elegans* wild type strain N2 Bristol and *unc*-119 *(ed3)*III were used in this work and these were provided by the *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota. The strains were maintained at 20°C on NGM plates seeded with *Escherichia coli* strains OP50 or HT115(DE3) following standard protocols (Brenner, 1974).

Bacterial Preparation and Induction

For feeding plates, Nematode Growth Medium (NGM) agar (Epstein and Shakes, 1995) was prepared including 25 µg/ml carbenicillin (carb) and 1mM IPTG (Kamath and Ahringer, 2003). 4.0 ml of agar was dispensed into 5cm plates (Nunc). Plates were allowed to dry inverted at room temperature before use. Bacteria (HT115 strain) expressing the gene of interest in glycerol stocks were streaked onto LB–agar plates including 50 µg/ml Amp (LBA) and 15 µg/ ml Tet and incubated over-night. Large innocula of bacteria were picked, inoculated into LB broth with 50 µg/ml Amp, and grown for 6–8 hours with shaking at 37^{o}C. Twenty (20) µl of 0.1M IPTG was added to 2ml of the incubated LBA (final concentration of 1mM) about 200µl of this culture was seeded/spread unto each of the above NGM derivative plates. The plates were dried thoroughly before being incubated overnight (~12–24 h) at room temperature in the dark to allow the bacteria to grow and to begin induction. *E. coli* transformation was performed with the $CaCl_2$ method.

Worm Synchronization, Aliquoting, and Feeding

N2 worms were cultured on standard NGM plates with OP50 *E. coli* prior to use in feeding experiments. Plates with large numbers of eggs were washed with M9 buffer (3 g of KH_2PO_4, 6 g of Na_2HPO_4, 5 g of NaCl, 1ml of 1M $MgSO_4$, in H_2O to 1 L). The solution was transferred to 15ml Eppendorf tubes, which were shaken vigorously by hand for 3–5 min to homogenize large worm particles. Eggs were pelleted by gentle centrifugation (2000 rpm) and were washed with M9 buffer. This is repeated 3 times. The supernatant was decanted leaving just about 500 µl on top of the pellet. 500 µl of a freshly prepared 2X 10% hypochlorite solution (1.25 ml of 2N NaOH, 1 ml of Aldrich sodium hypochlorite solution, 0.25 ml of dH_2O) was added and vortexed quickly. The mixture is incubated at room temperature for 2 min and immediately 10 ml of M9 solution was added. It was centrifuged at 3000 rpm for 2 min. The supernatant was discarded and the

last four steps were repeated 4 – 5 times by adding 10 ml of M9 on top of each wash. Finally, the supernatant was discarded leaving about 500 µl in the tube. The tube was then incubated at 20 ^{o}C with 120 rpm shaking over-night. The resulting synchronized L1-stage worms were then put on standard NGM plates with OP50 or HT115 (DE3) and were allowed to develop to adults.

Molecular Biology Techniques

Standard cloning and DNA plasmid extraction, electrophoresis, purification and precipitation were applied (Sambrook *et al*., 1989). Restriction endonucleases and DNA ligase (Fermentas) were used according to the manufacturer's specifications. PCR-amplification of the fragments of interest was performed with High Fidelity DNA Polymerase (Roche) according to the manufacturer's specification. *E. coli* transformation was performed with the $CaCl_2$ method.

RNA Interference and EtBr Assay

The RNAi experiments were performed using the feeding procedure described by Kamath (Kamath *et al*., 2003) with slight modifications (Addo *et al*., 2010). Feeding RNAi clones were purchased from the Ahringer RNAi library (Geneservice Limited) and sequenced. A single colony of HT115 (DE3) bacteria of interest (RNase III-deficient *E. coli* strain, carrying IPTG inducible T7-RNA polymerase) was first grown overnight at 37°C in LB-ampicillin. The bacteria were then seeded onto NGM plates with 1 mM IPTG, 25 µg/ml carbenicillin, and 50 µg/ml ampicillin (Kamath *et al*., 2003). For ethidium bromide (EtBr) plates, stock solution of 10mg/ml was used to prepare different concentrations as required and added before dispensing into the 5cm plates. Plates were then incubated at room temperature in a dark container for 48 h to allow the expression of the double-stranded RNA (dsRNA). Worms feeding on HT115 bacteria carrying the empty vector (L4440) were used as controls in all the experiments. Synchronized L1-stage N2 worms were placed onto NGM (RNAi) plates seeded with bacteria expressing the dsRNA gene of interest and were incubated for 72 h at 20°C. Four adult worms were independently picked up and transferred to fresh RNAi plates with or without different concentrations of ethidium bromide (EtBr). Worms were allowed to lay between 80 to 100 eggs before being removed. Eggs were immediately counted and the F1 progeny produced was analyzed after 3 and 4 days. At day 4, evaluation of the F1 progeny arrested at the L3 stage was compared to the number of adults on the same plate. The phenotype was scored as sensitive to RNAi and EtBr if more than 80% of worms were arrested at the L3-stage on plates containing 50 µg/ml of EtBr. A gene was considered as positive for a given phenotype if the same result was observed in at least two independent feeding experiments.

Investigation of mtDNA Integrity by Real-Time PCR (RT-PCR)

The efficiency and the specificity of the depleted transcripts in worms subjected to RNAi, was performed using a semiquantitative RT-PCR analysis of each of the identified mRNA level in the corresponding RNAi mutants. Briefly, total RNA was isolated from N2 worms fed on RNAi bacteria (HT115(DE3) strain) containing either the empty RNAi vector L4440 or the gene of interest dsRNA producing vectors with TRIzol Reagent (Invitrogen). RNA was treated with DNaseI (Invitrogen). First, single-strand cDNAs were synthesized (as described above) using

the reverse transcriptase SuperScript VILO™ and subsequently PCR amplified with the Expand High fidelity PCR system (Invitrogen) and PCR-amplified. The amplification of a 426-bp fragment of the *ama-1* cDNA was used as internal control. The amplified fragments were cloned into the pGEM-T easy vector (Promega). The inserts were sequenced on both strands. The integrity of mtDNA was checked by long-range PCR (Expand Long Template; Roche) using a pair of primers distant of 8561 bp (f-GAGCGTCATTTATTGGGAAG and r-CACAAAGGTCGACATATCAAC).

Bioinformatics Tools

BLAST information available in the *Saccharomyces* Genome Database was used to explore the *C. elegans* homologs for yeast genes at: http://www.yeastgenome.org/cgi-bin/blast-sgd.pl
MITOP2 was used to find the *C. elegans* homologs to yeast genes which are completely devoid of mtDNA (rho0) upon their inactivation: http://ihg.gsf.de/mitop2

Results and Discussion

A systematic screening by RNAi and EtBr exposure of 25% of the *C. elegans* genes encoding mitochondrial proteins was carried out. Among the 182 genes encoding mitochondrial proteins tested randomly, we found that inactivation of 25 of them lead to EtBr sensitivity (Table 1 - 4). These genes may represent genes important for the stability of the mtDNA in *C. elegans*. To confirm these results, silencing of these candidate genes were repeated again without EtBr to measure the animal's mtDNA content and the efficiency of gene silencing by Q-PCR and RT-Q-PCR respectively as described in the methods.

Most of these genes fall into four main categories of metabolism, protein synthesis, mitochondrial morphology and respiratory chain (oxidative phosphorylation):

Metabolism

The metabolic genes identified are expected since they encode metabolic proteins that have been already isolated as nucleoid components in different organisms (Table 1). Some of these genes like Aco1 (Aconitase), Ilv5 (acetohydroxyacid reductoisomerase), Ilv6 (acetolactate synthase), Lsc1 (alpha subunit of succinyl-CoA ligase), Pda1 (E1 alpha subunit of the pyruvate dehydrogenase (PDH) complex), and Pdb1 (E1 beta subunit of the pyruvate dehydrogenase (PDH) complex) have been found in *S. cerevisiae.* Some of them have been found as very important for mtDNA stability (Kucej and Butow, 2007) in these organisms. In Humans, AAT (aspartate aminotransferase), CPS1 (carbamoyl phosphate synthetase), CPT1A (carnitine palmitoyl transferase), DECR (2,4-dienoyl-CoA reductase), HADHA (hydroxyacyl dehydrogenase A), MDH (malate dehydrogenase), PDX3 (peroxide reductase), SHMT2 (serine hydroxymethyl transferase) have also been found.

Since there are very few overlap between metabolic proteins present in nucleoids of different species, which are supposed to be close to mtDNA, it is proposed that in *C. elegans* these metabolic genes also have a role on mtDNA stability perhaps as nucleoid components. For example, in this study, it was found that the gene *alh-3* (10-formyltetrahydrofolate dehydrogenase) (Table 1), which is orthologous to the human arginase 1 and genes encoding putative functions such as acyl-CoA thioester hydrolase and probable oxidoreductase is one of such genes.

Protein Synthesis

All the genes encoding mitochondrial ribosomal proteins tested in our screen led to EtBr sensitivity when inactivated (homologs of human MRPL12, MRPL4, MRPL47, MRPS14, MRPS18) (Table 2). However inactivating other components of the mitochondrial translation machinery such as some tRNA synthetases or the mitochondrial translational release factor 1 does not lead to EtBr sensitivity. These results suggest that in *C. elegans* mitochondrial ribosomes (large and small subunits) are important for mtDNA maintenance. In human, mutations in mitochondrial ribosomal protein MRPS22 and MRPS16 have been described in severe fatal respiratory chain dysfunction due to impaired translation of mitochondrial mRNAs. Southern blot analysis of muscle DNA revealed normal sized mtDNA but decreased mtDNA content, suggesting mtDNA depletion (Saada *et al*., 2007). Moreover, human MRPL12 has been shown to interact with POLRMT suggesting that MRPL12 can play a role in a potential novel regulatory mechanism that coordinates mitochondrial transcription with translation and/or ribosome biogenesis during mitochondrial gene expression (Wang *et al*., 2007). Interestingly, Addo *et al*., (2010) found that inactivation of POLRMT and MRPL12 (in this study) both lead to EtBr sensitivity evocative of mtDNA depletion suggesting that this interaction may also exist in *C. elegans* mitochondrial transcription.

Altogether, these results seem to indicate that part of the translation machinery, at least mitochondrial ribosomes, is needed for a normal mtDNA content in *C. elegans*.

Mitochondrial morphology

Four genes have been tested in this screen, two genes (*fis-1* and *fis-2*)(results not shown) homologous to the human FIS1 gene involved in the mitochondrial fission process, *eat-3* homologous to OPA1 involved in mitochondrial fusion and *letm-1* homologous to LETM1 (gene associated with Wolf-Hirschhorn syndrome: pre and postnatal growth retardation, severe mental retardation, and developmental delay with microcephaly) are important for the mitochondrial tubular shape. Only inactivation of eat-3 (OPA1) leads to a strong sensitive phenotype on EtBr suggesting mtDNA depletion (Table 3).

Both in human and in yeast, mutations in this gene lead to mitochondrial fragmention and loss of mtDNA. In *C. elegans eat-3* has been shown to be required for proper regulation of inner mitochondrial membrane fusion and normal mitochondrial oxidative phosphorylation function (Rolland *et al*., 2009). How this protein affects oxidative phosphorylation has not been determined however, these results strongly support that *eat-3* also plays a role on mtDNA maintenance in *C. elegans*.

Respiratory Chain – Oxidative Phosphorylation

Cytochrome P450

We found genes in the cytochrome P450 category which is involved in the respiratory chain complex and of the cytochrome P450 family (Table 4). Cytochromes P450 (CYPs) is a large and diverse group of enzymes that belong to the superfamily of proteins containing a heme cofactor and, therefore, are hemoproteins. Humans have 57 genes and more than 59 pseudogenes divided among 18 families of cytochrome P450 genes and 43 subfamilies. Most of them are mono-oxygenases that metabolize thousands of endogenous and exogenous chemicals. The substrates of CYP enzymes include metabolic intermediates such as lipids and

steroidal hormones, as well as xenobiotic substances such as drugs and other toxic chemicals.

In *C. elegans*, 80 cytochrome P450s NADPH-dependent mono-oxygenases exist. Among the 17 different Cytochrome P450 genes tested in our screen, inactivation of genes encoding Cytochrome P450 2C8 family (3) and one of the 3A7 family (Table 4) leads to EtBr sensitive phenotype. Finding this class of genes is not surprising if we consider that they can act on EtBr to avoid its toxic effect.

Oxidative Phosphorylation

Our results also showed one gene belonging to the mitochondrial ATP synthase (homolog of human ATP5F1, encoding the subunit b of Complex V), the subunit 18kDa of the NADH-ubiquinone oxidoreductase (NDUFS4, complex I) and the fumarate hydratase (FH) of the Krebs cycle (Table 4). This last enzyme is localized both in the cytosol and mitochondria and catalyzes the conversion of fumarate to malate. In human, mutations in this gene can cause fumarase deficiency and lead to an amazing variety of disease phenotypes, ranging from childhood encephalomyopathies, similar to those caused by recessive mutations of the mitochondrial respiratory chain and by some mtDNA mutations, to hereditary tumor formation (Bourgeron *et al*., 1994, Raimundo *et al*., 2008). Up to date, no studies on fumarase deficiency have addressed the question of mtDNA stability.

The NADH-ubiquinone oxidoreductase complex (complex I) is located in the inner mitochondrial membrane. NDUFS4 is not part of the catalytic core of complex I and its catalytic site is exposed to the matrix side of the inner mitochondrial membrane (Sardanelli *et al*., 1996). It is not directly involved in electron transport within complex I, but is thought to play an important role in the assembly and/or stability of the complex (Kruse *et al*., 2008) and may play a regulatory role because it can be phosphorylated (Papa *et al*., 2001). In human, mutations in this gene are implicated in Parkinson's disease and have been found in one case of early onset hypertrophic cardiomyopathy and encephalopathy. These results suggest that the catalytic activity of Complex It does not seem to be important for mtDNA activity but rather the correct assembly and /or stability of the complex.

Table.1 Human homologs of *Caenorhabditis elegans* metabolic proteins sensitive to Ethidium Bromide RNA interference (RNAi)

%L3 arrest	Ce name	Target gene	RNAi library	Human name	Human information	Gene category
90	x	C14F11.1a	X-3I16	GOT2	Aspartate aminotransferase, mitochondrial precursor1.3e-156 97.8%	METABOLISM
95	x	DC2.5	V-1E11	WWOX	Probable oxidoreductase, FOR II protein 2.8e-39 98.1%	METABOLISM
94	alh-3	F36H1.6	IV-5J04	ALDH1L2	10-formyltetrahydrofolate dehydrogenase 5.3e-275 99.5%	METABOLISM
98	x	K10H10.6	II-9M15	WWOX	Probable oxidoreductase, FOR II protein 6.3e-4295.9%	METABOLISM
99	x	T21F4.1	X-3I19	AGMAT	Splice Isoform 2 of Arginase 1 1.6e-29 67.9%	METABOLISM
90	x	T07D3.9a	II-1F09	ACOT9	Similar to Putative acyl-CoA thioester hydrolase CGI-162e-48 83.2%	METABOLISM

Table.2 Human homologs of *Caenorhabditis elegans* ribosomal proteins sensitive to Ethidium Bromide RNA interference (RNAi)

%L3 arrest	Ce name	Target gene	RNAi library	Human name	Human information	Gene category
100	x	B0035.16	IV-6E15	TRMU	tRNA(5-methylaminomethyl-2-thiouridylate)-methyltransferase 3.3e-68 97.9%	PROTEIN SYNTHESIS
99	x	B0261.4	I-2G22	MRPL47	39S ribosomal protein L47, mitochondrial precursor 2.5e-24 52.0%	PROTEIN SYNTHESIS
100	x	T01E8.6	II-7O21	MRPS14	Mitochondrial 2S ribosomal protein S14 5.4e-18 53.3%	PROTEIN SYNTHESIS
96	x	T14B4.2	II-5M13	MRPS18C	28S ribosomal protein S18c, mitochondrial precursor 3.2e-21 69.1%	PROTEIN SYNTHESIS
100	x	T23B12.2	V-6E01	MRPL4	Mitochondrial ribosomal protein L4, isoform a 7e-54 95.3%	PROTEIN SYNTHESIS
100	x	W09D10.3	III-5P17	MRPL12	39S ribosomal protein L12, mitochondrial precursor 5e-26 79.2%	PROTEIN SYNTHESIS

Table.3 Human homologs of *Caenorhabditis elegans* mitochondrial morphology protein sensitive to Ethidium Bromide RNA interference (RNAi)

%L3 arrest	Ce name	Target gene	RNAi library	Human name	Human information	Gene category
100	eat-3	D2013.5	II-6L23	OPA1	Optic atrophy 1 isOfOrm 83.5e-239 83.6%	MORPHOLOGY

Table.4 Human homologs of Caenorhabditis elegans Respiratory chain proteins sensitive to Ethidium Bromide RNA interference (RNAi)

%L3 arrest	Ce name	Target gene	RNAi library	Human name	Human information	Gene category
100	asb-2	F02E8.1	X-2N13	ATP5F1	ATP Synthase chain, mitochondrial precursor 4.9e-11 66.6%	RESP. CHAIN OXID. PHOSPHORYLA.
96	lpd-5	ZK973.10	I-1L24	NDUFS4	NADH-ubiquinone oxidoreductase 18 kDa subunit, mitochondrial precursor 4..3e-35 64.3%	RESP. CHAIN OXID. PHOSPHORYLA.
100	fum-1	H14A12.2a	III-3P22	FH	Fumarate hydratase, mitochondrial precursor 4.2e-189 97.8%	RESP. CHAIN OXID. PHOSPHORYLA.
92	cyp-33B1	C25E10.2	V-6I24	CYP2C8	Cytochrome P450 2C8 2.2e-61 95.2%	RESP. CHAIN OXID. PHOSPHORYLA.
91	cyp-13B1	F02C12.5a	X-6E10	CYP3A7	Cytochrome P450 variant 3A7 2.8e-39 69.3%	RESP. CHAIN OXID. PHOSPHORYLA.
93	cyp-33c5	F41B5.3	V-2K09	CYP2C8	Cytochrome P450 2C8 5.8e-68 93.3%	RESP. CHAIN OXID. PHOSPHORYLA.
98	cyp-35A5	K07C6.5	V-3K04	CYP2C8	Cytochrome P450 2C8 1.2e-53 93.9%	RESP. CHAIN OXID. PHOSPHORYLA.

ATP5F1 or *C. elegans asb-2* encodes the b subunit of the membrane-bound F_0 proton channel portion of the mitochondrial ATP synthase. This gene has a paralog *asb-1* and large-scale RNAi experiments indicate that asb-2 activity is involved in larval development as well as for normal body morphology, lifespan, and growth rate, whereas asb-1 activity is needed for embryonic viability and for normal proliferation of germline cells, specifically their progression to meiosis (Simmer at al., 2003; Rual *et al*., 2004).

The mitochondrial ATP synthase is a membrane-bound protein complex that catalyzes the formation of ATP from ADP and inorganic phosphate. It has been shown, in yeast, that subunit b, required for ATP synthesis, contributes to the oligomerization of the complex. It is involved in the stability of supramolecular species of yeast ATP synthase in the mitochondrial membrane (Weimann *et al*., 2008). In yeast a deletion mutant of *ATP7* (subunit d), the catalytic portion F_1 was loosely bound to the membrane part and the mitochondrially encoded hydrophobic subunit 6 (subunit *a* of the F_0) was not present (Norais *et al*., 1991).

All in all, these results also point to an important role of the ATP synthase in mtDNA maintenance in *C. elegans*. Whether these proteins or the ATP synthase play a direct role on mtDNA stability in addition to its role in synthesis of ATP is very interesting and deserves more experiments.

In conclusion, our work has enabled us to identify certain genes that may have a role on mtDNA stability perhaps as nucleoid components. The human orthologs of these genes as presented in this study should therefore be considered as reservoir of new genes which will represent a helpful tool and knowledge for mitochondrial diseases, as they would be considered as candidate genes for patients.

Acknowledgement

The authors acknowledge the French Government through its Embassy in Ghana for the financial support extended to this study.

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How to cite this article:

Matthew Glover Addo, Raynald Cossard, Damien Pichard, Kwasi Obiri-Danso, Agnes Rötig and Agnes Delahodde. 2016. Identification of New Genes Involved in mtDNA Maintenance in *Caenorhabditis elegans* that could Represent Candidate Genes for Mitochondrial Diseases. *Int.J.Curr.Microbiol.App.Sci.* 5(6): 179-189.
doi: http://dx.doi.org/10.20546/ijcmas.2016.506.022