

DEHYDROGENASE'S 4L SUBUNIT TRANSLATION AND POLYPEPTIDIC CHAIN CHARACTERISATION FOR THE ESTABLISHED HAPLOTYPE FOR INDIVIDUALS OF *CYPRINUS CARPIO* L., (1785) (*CYPRINIDAE*) SPECIES, SAMPLED FROM TWO POPULATIONS – MOVILENI AND IASI

LUCIAN D. GORGAN^{1*}

Keywords: ND4L dehydrogenase, *Cyprinus carpio* L.,

Abstract: In present experiment, our purpose was translation and characterization of the polypeptidic chain, specific for the haplotypes of the gene which determines dehydrogenases' 4L sub-unit synthesis, for individuals of *Cyprinus carpio* L. species, with origin in two different populations – Movileni and Iași.

INTRODUCTION

The NADH:ubiquinone oxidoreductase (Complex I), provides the input to the respiratory chain from the NAD-linked dehydrogenases of the citric acid cycle. The complex couples the oxidation of NADH and the reduction of ubiquinone, to the generation of a proton gradient which is then used for ATP synthesis. The complex occurs in the mitochondria of eukaryotes and in the plasma membranes of purple photosynthetic bacteria, and the closely related respiratory bacteria. The close homology of sequences, function, and prosthetic groups shows a common ancestry. Subunit 1 is one of 7 mitochondrial DNA (mtDNA) encoded subunits (MTND1, MTND2, MTND3, MTND4, MTND4L, MTND5, MTND6) included among the approximately 41 polypeptides of respiratory Complex I (NADH:ubiquinone oxidoreductase,) (Shoffner and Wallace, 1995; Arizmendi et al., 1992; Walker et al., 1992; Anderson et al., 1981; Attardi et al., 1986; 14,13:Chomyn et al., 1985, 1986; Wallace et al., 1986; Oliver and Wallace, 1982; Wallace et al., 1994).

Complex I is the first step in the electron transport chain of mitochondrial oxidative phosphorylation (OXPHOS) and is located within the mitochondrial inner membrane. It accepts electrons from NADH and transfers them, through a series of electron carriers, to ubiquinone (Coenzyme Q10). The internal electron carriers of complex I include flavin mononucleotide (FMN) and 6 iron-sulfur clusters designated N-1a, N-1b, N-2, N-3, N-4, and N-5 (Ohnishi, 1979; Ragan, 1987). Complex I can be subdivided into 3 main fractions: the flavoprotein fragment, the iron-protein fragment, and the hydrophobic protein fragment (Ragan, 1987). The flavoprotein fragment contains the FMN, 6 of the iron atoms, and 3 polypeptides (51, 24, and 10 kD) (Galante and Hatefi, 1979; Ragan et al., 1982). The NADH-binding site and FMN have been assigned to the 51-kD polypeptide (Chen and Guillory, 1981). The iron-protein fragment contains 9 or 10 iron atoms (Ragan et al., 1982), and a 15-kD protein from this fragment appears to be the ubiquinone-binding protein involved in electron transfer to ubiquinone (Suzuki and Ozawa, 1986). The MTND6 protein may also be located in the iron-protein fragment (Chomyn et al., 1986). The hydrophobic protein fragment contains the iron-sulfur centers that are the likely electron donors to ubiquinone (Ohnishi et al., 1985; Ohnishi et al., 1974). Of the 7 mitochondrial DNA Complex I genes, the gene products for MTND1, MTND3, and MTND4L have been localized to the hydrophobic protein fragment (Ragan, 1987), and the MTND2, MTND4, and MTND5 gene products probably reside there also.

NADH dehydrogenase has 7 sub-units on the mtDNA's surface. 4L sub-unit was chosen for amplification and sequencing, because it's considered to be a highly conservative region.

Other studies based on sequences of ND4L for other fish species, were recorded for *Acipenseriformes*, endemic Chinese species and species from Europe and North America as well. In recent years, the use of mtDNA *ND4L* and *ND4* genes for phylogenetic analysis has drawn attention.

MATERIALS AND METHODS

For *Cyprinus*' genera species, towards gene amplification, a modified reaction mixture obtained by duplicating *Taq* polymerases' quantity from 0,3μl to 0,6μl, was used. Towards amplifying this specific segment, two degenerated primers were used: L10420 for the direct strand and H10720 for the reverse strand (Figure 1 and Figure 2).

The gene's amplifying reaction took place at 51°C as primer's aligning temperature, and a number of 40 replication cycles (Figure 3).

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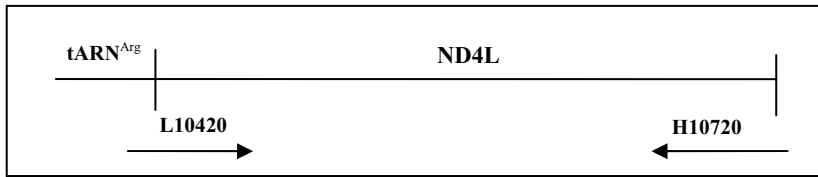


Figure 1 Primers' position in ND4L amplifying

L10420 5'-AAYAARAYCNTTGATTTCGRCTCA-3'
 H10720 5'-TCYGTGCCRTGYGTYCGNGC-3'

Figure 2 Primers' structure in ND4L amplifying at cyprinids

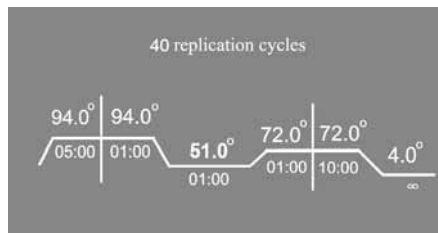


Figure 3 Graphic representation of PCR stages for ND4L

Towards sequencing the codifying gene for NADH 4 L sub-unit's synthesis information, for all analyzed species, ginogenetic and hybrid individuals, we used the same primers: L10420 for the direct chain and H10720 for the reverse chain in 10μM concentration. The sequencing reaction program for species of *Cyprinus* genera had a primer's aligning temperature of 47°C in 30 replication cycles (Figure4).

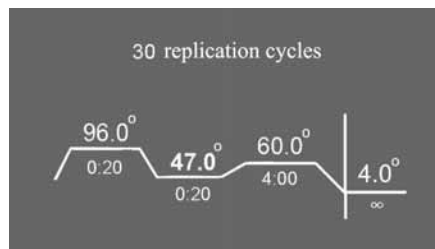


Figure 4 Sequencing reaction conditions for ND4L for *Cyprinus* genera species

Sequence's primary process as fluorograms, and correction were made using CEQ2000 program by BECKMAN COULTER, and the obtained data was exported as text, towards alignment.

Binding the direct and reverse chains for each individual, was made using ESEE 32 (The Eyeball Sequence Editor) program, through alignment with primer's sequences and cutting out the two chains, followed by the association in an unique chain.

Aligning all the gene's sequences, resulted from different individuals, (individuals from different populations, obtained by ginogenesis or hybrids) was carried out through ClustalW (Thompson et. al., 1994) și Clustal V (Higgins și Sharp, 1989; Higgins, 1994; Wheeler, 2001) methods, using the MegAlign module, from DNASTAR 5 (trial version) program.

Based on the observed differences, between the analyzed sequences, the characteristic haplotype for the two populations was established. Based on this haplotype's sequence, translation was later established, using a mitochondrial

genetic code for vertebrates (Figure 5 and Figure 6). Based on the obtained polypeptidic chain after translation, the total number of amino acids, their type, frequency and percents from total weight were quantified.

| | U | C | A | G | |
|---|--|--------------------------------------|--|---|------------------|
| U | UUU } Phe UUC } UUA } Leu UUG } | UCU } Ser UCC } UCA } UCG } | UAU } Tyr UAC } UAA Stop UAG Stop | UGU } Cys UGC } UGA Stop UGG Trp | U C A G |
| C | CUU } CUC } Leu CUA } CUC } | CCU } Pro CCC } CCA } CCG } | CAU } His CAC } CAA } Gln CAC } | CGU } Arg CGC } CGA } CGG } | U C A G |
| A | AUU } AUC } Ile AUA } AUG } Met | ACU } Thr ACC } ACA } ACG } | AAU } Asn AAC } AAA } Lys AAG } | AGU } Ser AGC } AGA } Arg AGG } | U C A G |
| G | GUU } GUC } Val GUA } GUG } | GCU } Ala GCC } GCA } GCG } | GAU } Asp GAC } GAA } Glu GAG } | GGU } GGC } Gly GGA } GGG } | U C A G |

Figure 5 Standard genetic code

| | U | C | A | G | |
|---|--|--------------------------------------|--|--|------------------|
| U | UUU } Phe UUC } UUA } Leu UUG } | UCU } Ser UCC } UCA } UCG } | UAU } Tyr UAC } UAA Stop UAG Stop | UGU } Cys UGC } UGA Trp UGG Trp | U C A G |
| C | CUU } CUC } Leu CUA } CUG } | CCU } Pro CCC } CCA } CCG } | CAU } His CAC } Gln CAA } | CGU } Arg CGC } CGA } CGG } | U C A G |
| A | AUU } AUC } Ile AUA } AUG } Met | ACU } Thr ACC } ACA } ACG } | AAU } Asn AAC } AAA } Lys AAG } | AGU } Ser AGC } AGA Stop AGG Stop | U C A G |
| G | GUU } GUC } Val GUA } GUG } | GCU } Ala GCC } GCA } GCG } | GAU } Asp GAC } GAA } Glu GAG } | GGU } GGC } Gly GGA } GGG } | U C A G |

Figure 6 Mitochondrial genetic code

RESULTS AND DISCUSSIONS

From the 21 sequences alignment, no differences were recorded at this mtDNA level, between individuals of the two studied populations (Larga-Jijia – Movileni and Iași). Further more, we can conclude that ND4L is a conservative gene, with a reduced variability. However, comparing the Cy GB sequence with the general halotype of the two populations, we can determine the existence of three differences. Thereby, for the sequences of the two populations, one transition took place in 165 position (adenine substitution with guanine), another transition occurred in 180 position (thymine substitution with cytosine), and one transversion in 258 position (adenine substitution with cytosine).

As fore-cited, we can conclude that a new haplotype, characteristic for the two populations, was found (Cy01IM).

Translation took place based on the mitochondrial genetic code for vertebrates, using EditSeq module from DNA Star (limited edition) program; the obtained polypeptidic chain is presented in Figure 7.

**MTPVHFSFSS AFILGLMGLA FHRTHLLSAL LCLEGMMLSL FIALALWALQ FESTGFSTAP
MLLAFSACE ASTGLALLVA ARTHGT**

Figure 7 ND4L polypeptidic chain, characteristic for *Cyprinus carpio* species
Cy01IM haplotype

After translation, we can observe that the entire polypeptidic chain consists of 87 amino acids. From Table 1 and Figure 8 one can notice that leucyene has the highest frequency (21,84%), and that glycine and tryptophan have the lowest frequency (1,15%). We can also observe that aspartic acid, lysine, asparagine and tyrosine are missing.

Three of the 87 amino acids are acidic, 2 are basic, 20 are polar and 45 amino acids are hydrophobic (Table 2). The polypeptidic chain has an electric charge of -0,49 (for ph=7) and an isoelectric point of 6,73 (Table 2 and Figure 9).

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Table 1 Polypeptidic chain composition

| Amino acids | | Number | Percents from total weight (%) | Frequency (%) |
|-------------|------|--------|--------------------------------|---------------|
| Symbol | Name | | | |
| A | Ala | 13 | 9,99 | 14,94 |
| C | Cys | 2 | 2,23 | 2,30 |
| D | Asp | 0 | 0,00 | 0,00 |
| E | Glu | 3 | 4,19 | 3,45 |
| F | Phe | 8 | 12,73 | 9,20 |
| G | Gly | 6 | 3,70 | 6,90 |
| H | His | 4 | 5,93 | 4,60 |
| I | Ile | 2 | 2,45 | 2,30 |
| K | Lys | 0 | 0,00 | 0,00 |
| L | Leu | 19 | 23,25 | 21,84 |
| M | Met | 5 | 7,09 | 5,75 |
| N | Asn | 0 | 0,00 | 0,00 |
| P | Pro | 2 | 2,10 | 2,30 |
| Q | Gln | 1 | 1,39 | 1,15 |
| R | Arg | 2 | 3,38 | 2,30 |
| S | Ser | 9 | 8,48 | 10,34 |
| T | Thr | 8 | 8,75 | 9,20 |
| V | Val | 2 | 2,14 | 2,30 |
| W | Trp | 1 | 2,01 | 1,15 |
| Y | Tyr | 0 | 0,00 | 0,00 |
| B | Asx | 0 | 0,00 | 0,00 |
| Z | Glx | 0 | 0,00 | 0,00 |
| . | Ter | 0 | 0,00 | 0,00 |

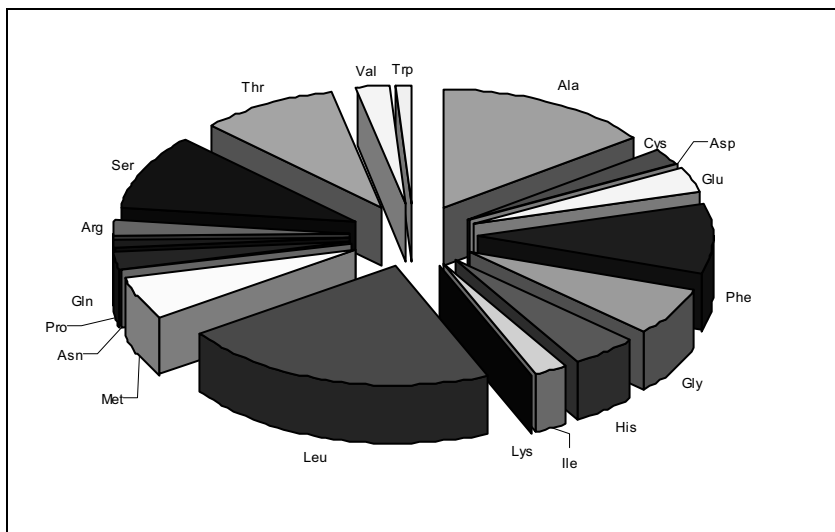


Figure 8 Amino acids' percents

Table 2 Polypeptidic chain characterization

| Molecular weight (Daltons) | Number of amino acids | Amino acids | | | | Isoelectric point | Electric charge for pH=7 | Concentration for E=1 and λ=280nm |
|----------------------------|-----------------------|--------------|---------------|-------------|-------|-------------------|--------------------------|-----------------------------------|
| | | Highly basic | Highly acidic | Hydrophobic | Polar | | | |
| 9247,07 | 87 | 2 | 3 | 45 | 20 | 6,736 | -0,479 | 1,56 |

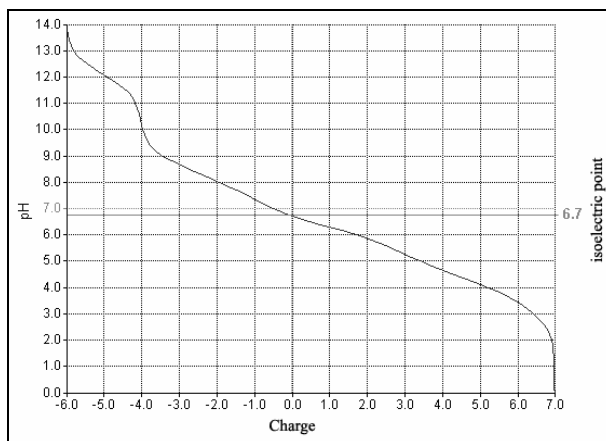


Figure 9 ND4L polypeptidic chain titration curve, for *Cyprinus carpio* species Cy01IM haplotype

Subsequently, the protein structure was analyzed, (Figure 10) and alpha regions, beta regions and turn regions have been determined using two methods Garnier-Robson (Garnier et al., 1978) and Chou-Fasman (Chou and Fasman, 1978); hydrophilicity plot graphic representation (Kyte and Doolittle, 1982), flexible regions (Karplus și Schultz, 1985), antigenic index (Jameson and Wolf, 1988) and surface probability plot (Emini et. al., 1985).

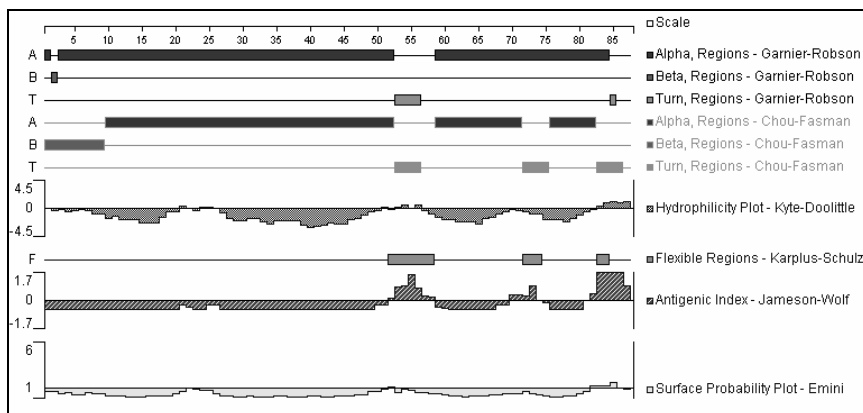


Figure 10 Polypeptidic chain structure analysis

The polypeptidic chain structure analysis shows that there are no major differences between Garnier-Robson and Chou-Fasman methods, regarding the alpha helical regions; thereby, in both methods, we determined the existence of 3 alpha helical regions, with a surface partial superposition comparative between methods. Regarding the beta folded regions, for both methods, we can detect the existence of one single beta folded region, at the starting point of the polypeptidic chain, with the distinction given by those regions' surface size differences, determined based on the two methods.

Regarding the modulation regions, for the first method (Garnier-Robson), 2 such zones are given, while by Chou-Fasman method 3 regions can be detected; the difference is that in the second method, they are presented as regions with larger surfaces.

Based on the hydrophobicity graphic, made after the Kyte-Doolittle model (Kyte and Doolittle, 1982), 6 hydrophobic and 6 hydrophilic zones were determined.

From the antigenic index graphic, calculated based on the Jameson-Wolf model (Jameson and Wolf, 1988), 3 regions with antigenic potential were observed.

CONCLUSIONS

The polypeptidic chain, for dehydrogenases' 4L sub-unit, consists of 87 amino acids, from which leucine has the highest frequency, (21,84%), while glycine and tryptophan have the lowest frequencies (1,15%).

The polypeptidic chain has three alpha regions and one beta region.

Hydrophilicity plot show five hydrophobic regions formed by 45 hydrophobic amino acids and 4 small hydrophilic regions.

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1 – “Alexandru Ioan Cuza” University of Iasi,
Faculty of Biology, Department of Genetics

* E-mail: lucian.gorgan@uaic.ro

