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The revelation of expressing region in the processed ceruloplasmin gene in human genome by biocomputational and biochemical methods

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Abstract

Annotation: Translation in all open reading frames (ORF) of human ceruloplasmin (Cp) pseudogene revealed the only translating sequence of 984 nucleotides. The amino acid sequence contains a signal peptide for mitochondrial protein import at N-terminus. The predicted protein without taking the signal peptide into consideration has 92% identity to the corresponding Cp fragment. It contains 20 amino acid substitutions, 8 of them are significant. There is His-X-His motif in the center of a molecule that is typical for copper containing oxidases. Potential copper-binding site appears as a result of the substitution P923H along human Cp sequence. Cp pseudogene transcription product was found in the cultured human cell lines HepG2 and HuTu 80 using RT-PCR strategy. Cp polypeptides with molecular weight of nearly 30 kDa were found in mitochondria of HuTu 80 cells. The possible biological role of mitochondrial Cp is under discussion.

Keywords: Ceruloplasmin; Ceruloplasmin pseudogene expression; Mitochondria

1. Introduction

Ceruloplasmin (Cp, E.C. 1.16.3.1) is a multicopper oxidase of vertebrates, it plays a central role in iron metabolism and copper transport [1,2]. This polyfunctional protein whose functions depend on many parameters (cell type, period development, localization) [3], has several soluble and membrane bound isoforms [4–9]. So far only two Cp-mRNA isoforms are described. They are formed from a common Cp gene transcriptional product in the process of alternative splicing [9]. We used biocomputational strategy for searching for homologous to Cp functional sequences in GenBank database and revealed a potential mitochondrial Cp-like protein encoded by human Cp pseudogene. The experimental data supporting the existence of mitochondrial Cp are also presented.

2. Experimental

Cultured cells of HepG2 line, primarily obtained from human hepatoblastoma, and HuTu 80 line, primarily obtained from human small intestine adenocarcinoma, were used. Cells were cultured in DMEM, containing 2 mM glutamine, 0.1 mg/ml ampicillin, supplemented with 10% fetal calf serum. Subcellular fractions enriched with plasma membranes, mitochondria and intracellular membranes were isolated using differential centrifugation from cellular homogenate in 0.25 M sucrose, 100 mM KCl, 8 mM $MgCl_2$, 10 mM tris–HCl, pH7,4, 5% β-mercaptoethanol, 0.5 μl/ml (in 1:1000 ratio) protease inhibitors cocktail ("Sigma", CIIIA). HepG2 and HuTu 80 RNA was extracted with RNAqueous™ (Ambion, USA) according to the manufacturer's instructions. RT-PCR was performed with 1 μg RNA, oligo d(T)18 primer (1 μM), 500 μM dNTPs ("Promega", USA), 5× RT-buffer and M-Mlv reverse transcriptase (200 U, Amplisens, Russia) at 37 °C for 1 h. 1 μl of RT-sample was added to PCR mix (1.5 mM $MgCl_2$, 200 μM dNTPs, 5× PCR buffer, 1.5 U Taq DNA polymerase) with 0.5 μM of each primer. Western blots were carried out in

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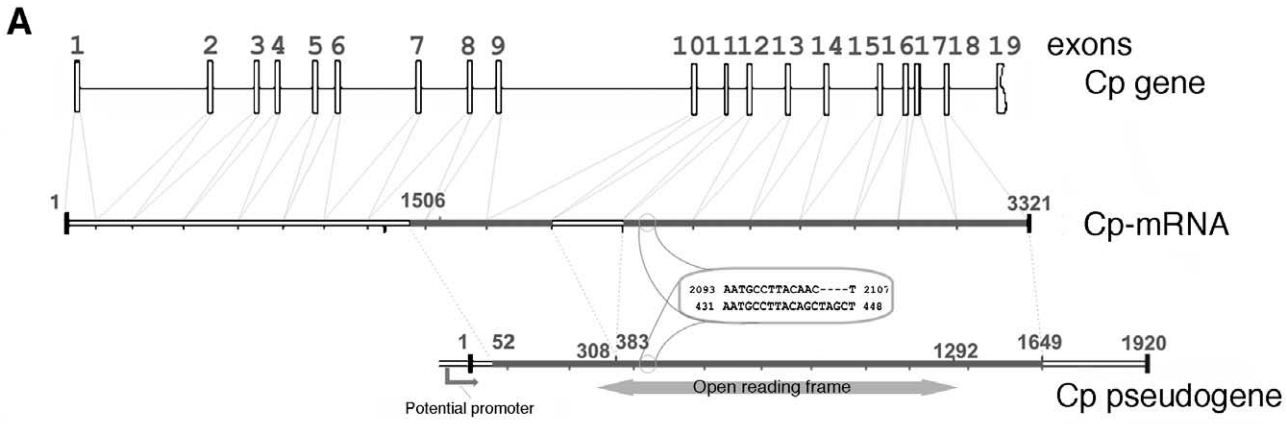

B

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pseudo Cp MRMRVYLLEDNIRMFTTAPDQVDKQMKTFRNLIKCTGLLMLNALQL---------------------------------------------------------- 45
      Cp DENESLLLEDNIRMFTTAPDQVDKEDEDFQESNKMHSMNGFMYGNQPGLTMCKGDSVVWYLFSAGNEADVHGIYFSGNTYLWRGERRDTANLFPQTSLTLHMWPD 690
         ******************    *

pseudo Cp ------------DHYTGGMKQKYTVNQCRWQSEDSTFYLGERTYYIAAVEVEWDYSPQREWEKELHHLQEQNVSNAFLDKGEFYIGSKYKKVVYRQYTDCTFRIP 140
      Cp TEGTFNVECLTTDHYTGGMKQKYTVNQCRRQSEDSTFYLGERTYYIAAVEVEWDYSPQREWEKELHHLQEQNVSNAFLDKGEFYIGSKYKKVVYRQYTDSTFRVP 775
                ***************** ********************************************************************** *** *

pseudo Cp VERKAEEEHLGILALQLHADVGDKVKIIFKNMTTRPYSIHAHGVQTESSTFIPALPGETLTYLWKIPERSGAGTEDSACIPWAYYSTVDQVKDLYSGLIGPLIVC 245
      Cp VERKAEEEHLGILGPQLHADVGDKVKIIFKNMATRPYSIHAHGVQTESSTVTPTLPGETLTYVWKIPERSGAGTEDSACIPWAYYSTVDQVKDLYSGLIGPLIVC 860
         *************  ***************** ********************************************************************

pseudo Cp TESSTFIPALPGETLTYLWKIPERSGAGTEDSACIPWAYYSTVDQVKDLYSGLIGPLIVCRRHYLKVFNPRKKLEFTLLFLVFDENESWYLDDNIKTNSDHPKKV 290
      Cp TESSTVTPTLPGETLTYVWKIPERSGAGTEDSACIPWAYYSTVDQVKDLYSGLIGPLIVCRRPYLKVFNPRRKLEFALLFLVFDENESWYLDDNIKTYSDHPEKV 945
         *****  * ******** ********************************************** ******** **** ******************** **** **

pseudo Cp NKDDEEFIESNKMHAVNGRMFGNPQGLTMHMGDEANGR------------------------------------ 328
      Cp NKDDEEFIESNKMHAINGRMFGNLQGLTMHVGDEVNWYLMGMGNEIDLHTVHFHGHSFQYKHRGVYSSDVFDIFP 1020
         *************** ******* ****** *** *
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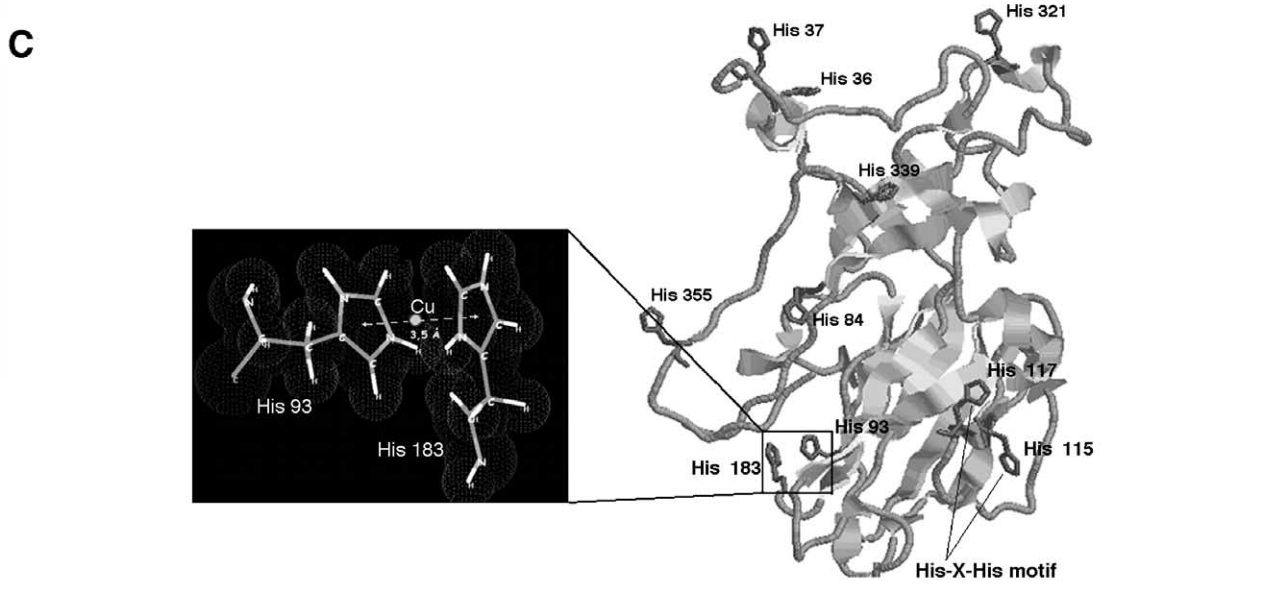

Fig. 1. Comparison of nucleotide and amino acid sequences of Cp and Cp pseudogene. (A) Above: exon/intron structure of human Cp gene. Vertical rectangles are exons. Below: mRNAs of Cp and *pseudo*Cp. Homologous regions are overshadowed; 4-nucleotide insertion is shown in a frame. (B) SPMPI is marked out with light font, His-X-His motif and histidines forming three dimensional sandwich are shown in a frame. (C) A model of the predicted mature expression product of Cp pseudogene. The model is built on the basis of corresponding region of human Cp molecule, obtained by the method of X-ray analysis [10], by replacement of non-corresponding amino acids and subsequent energy minimization using Polak-Ribiere algorithm (HyperChem 7.0.1. program). Graphical representation of a molecule was made using RasMol 2.7.1. program. Incut: sandwich with the bilateral symmetry of imidazole rings of His93 and His183, distance between the rings is 3.5Å.