Correlation Between Molecular Heterogeneity of Ceruloplasmin mRNA and Ceruloplasmin Isomers Synthesized in Rat Liver

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Molecular heterogeneity of ceruloplasmin mRNA is studied in rat liver polyribosomes. Blot hybridization of RNA with cDNA reveals three molecular forms of ceruloplasmin mRNA differing in the chain length and structure of the 3'-regions. A correlation between these mRNAs and three isoforms of ceruloplasmin, which perform different functions in the copper transport, is established.

Key Words: *mRNA; ceruloplasmin; isoforms; rat liver*

Copper is a constituent of more than 30 metalloproteins which control important functions of the eukaryotic cell [5]. Cytotoxicity of copper ions (CI) is prevented by their packing into protein molecules, providing inter- and intracellular transport of CI. Both deficiency and excess of CI lead to imbalance of vital functions. The balance of CI in the body is controlled by expression of genes encoding for the copper transport proteins [6]. In mammals, copper homeostasis at the organ level is controlled by the liver. Molecular and cellular mechanisms of this control are unclear. Ceruloplasmin (CP), a Cu-containing glycoprotein, is the major transporter of CI. The CP gene is expressed in numerous organs and is tissue-specific [1,3,14]. Previously, we demonstrated molecular heterogeneity of CP- and CP-like proteins synthesized in different organs: they have different physicochemical properties and perform different functions in copper metabolism [2-4]. Three CP isomers are synthesized in hepatocytes: serum CP (132 kD, this protein is secreted into circulation), bile CP (200 kD), and intracellular nonsecretory CP-like protein (50 kD). *In vivo* studies showed that these proteins are independent molecular forms of CP [2-4]. However, CP mRNA (3400-3700 nucleotides) coding for serum CP (132 kD) predominates in the poly(A)$^+$-RNA of rat polyribosomes [8,9]. In this study we analyzed heterogeneity of the ceruloplasmin mRNA in rat liver polyribosomes.

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MATERIALS AND METHODS

Outbred male albino rats weighing 120 g were used. Reagents were from Sigma, salts were from Merck, restrictases were from Amersham, and nitrocellulose filters (pore diameter 45 μ) were from Serva. An RM-1 X-ray film was used. The total polyribosome RNA fraction was isolated from rat liver as described [12]. Ceruloplasmin cDNA cloned in pCplg4 and pCp9B plasmids was used for blot hybridization. These plasmids carry the CP mRNA sequences (nucleotides 421-3715 and 72-3000) [8]. Fractionation of RNA in agarose gel with formaldehyde, isolation of plasmid DNA, incubation with restrictases, fractionation of DNA fragments in agarose gel, elution, labeling with [α-^{32}P]dATP in DNA-polymerase reaction with multiple statistical primers, and blot hybridization were performed by the methods [13]. Ceruloplasmin cDNA fragments 2100, 700, and 500 bp complementary to various segments of the characterized rat CP mRNA were used in experiments (Fig. 1).

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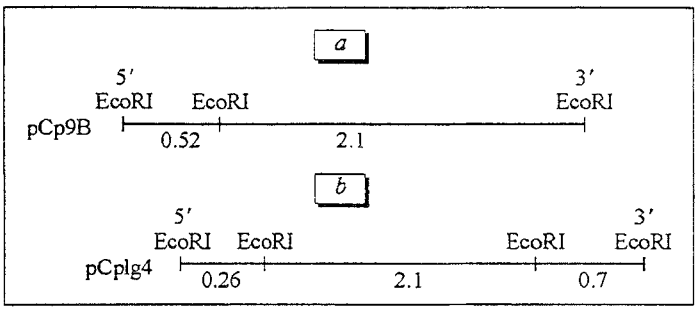

Fig. 1. Clones of CP cDNA and positions of fragments employed in blot hybridization with CP mRNA. *a*) CP cDNA insertion into pCp9B plasmid (nucleotides 72-3000 in CP mRNA); *b*) CP cDNA insertion into pCplg4 plasmid (nucleotides 421-3715 in CP mRNA).

RESULTS

The poly(A)$^+$-RNA fraction isolated from human liver contains numerous molecular forms of CP mRNA that can be identified by hybridization with full-length CP cDNA [11] or by comparative analysis of nucleotide sequences of cDNA clones [15]. No reliable heterogeneity of CP mRNA was revealed in rats [8]. To clarify this issue we employed total polysomal RNA (without fractionation into poly(A)$^+$ and poly(A)$^-$, assuming that under the stringent conditions of chromatography on oligo(dT)-cellulose mRNA molecules with short poly(A) fragments are isolated with ribosomal RNA. Full-length CP cDNA and its fragments were used as selective probes to detect structural differences in the CP mRNA. The results of these experiments are summarized in Table 1. Classic CP mRNA (3400 nucleotides) was revealed with all probes. DNA fragments 520 and 2100 bp, which are complementary to the 5'-region of CP mRNA, revealed three molecular forms of CP mRNA: 4100, 3400, and 1800 nucleotides in length (Fig. 2). RNA degradation can be ruled out, since only native polysomal RNA characterized by discrete electrophoretic zones corresponding to 28S- and 18S-rRNA (molar ratio 1:1) was used in these experiments. Therefore, it can be suggested that the true molecular heterogeneity of CP mRNA has been revealed, and the molecular forms of this mRNA differ not only in the chain length but also in nucleotide sequences in the 3'-region, having similar 5'-regions. Presumably, different CP mRNA were formed as a result of alternative splicing of pre-mRNA, a primary transcript of the CP gene. In order to confirm this hypo-

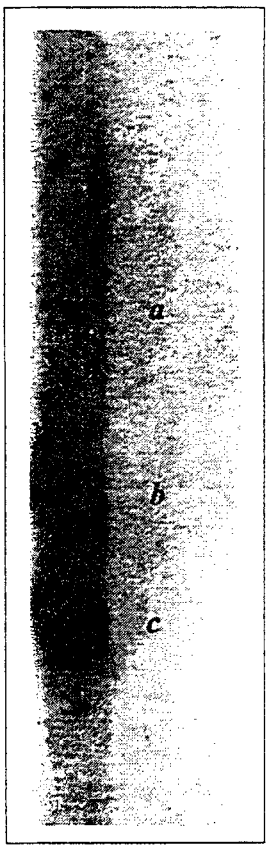

Fig. 2. Blot hybridization of rat liver polyribosomal RNA with full-length [^{32}P]CP cDNA (clone pCp9B). Ceruloplasmin isoforms 4100 (*a*), 3400 (*b*), and 1800 (*c*) nucleotides in length.

TABLE 1. Molecular Forms of CP mRNA Isolated From Rat Liver Polyribosomes

[^{32}P]DNA probe	Size of revealed mRNA, nucleotides		
	CP 200 kD	CP 132 kD	CP 50 kD
Full-length CP cDNA	4100	3400	1800
2100 bp cDNA fragment	4100	3400	1800
520 bp cDNA fragment	4100	3400	
700 bp cDNA fragment		3400	1800

Note. For topography of cDNA fragments see Fig. 1. The mRNA size was calculated by its relative mobility in 1% agarose gel under denaturing conditions [10]. 28S- and 18S-rRNA served as internal markers.

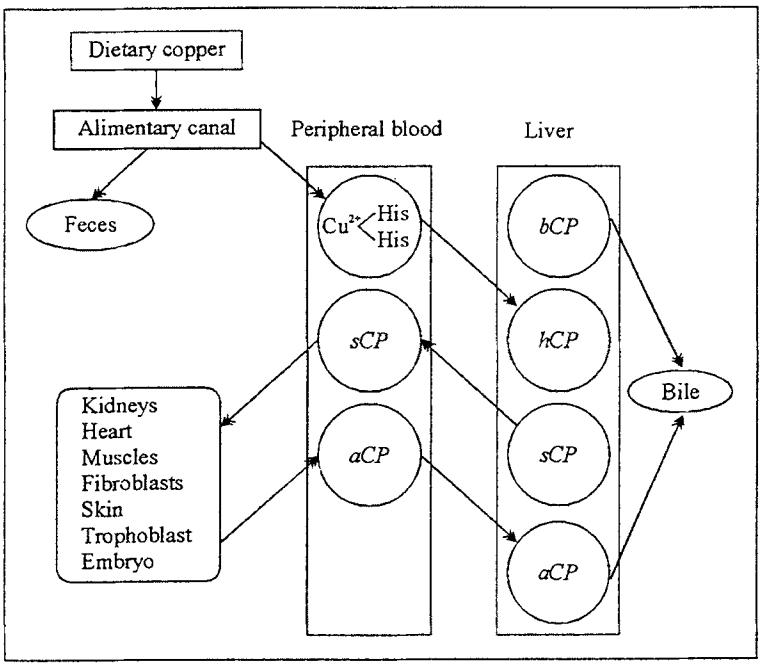

Fig. 3. Role of multiple molecular forms of ceruloplasmin in the regulation of accumulation, distribution, and excretion of copper in rats. *His*) histidine; *sCP*) serum ceruloplasmin; *hCP*) intracellular ceruloplasmin of hepatocytes; *bCP*) bile ceruloplasmin; *aCP*) blood asialoceruloplasmin internalized by hepatocytes.

thesis, clones of the cDNAs of individual CP mRNAs should be compared. In a first approximation, the described forms of CP mRNA correspond to three CP isoforms synthesized in rat liver and characterized in our previous investigations [2-4]. It is likely that at the molecular level these isoforms provide accumulation, distribution, and excretion of CI. A scheme illustrating the participation of the three forms of CP in the CI turnover in mammals is shown in Fig 3. The scheme reflects the rate of synthesis, dynamics, secretion, and distribution of CP in internal organs and intracellular compartments of rat hepatocytes [7]. Part of dietary CI is excreted, while CI absorbed in the intestine enter the circulation and are transported to the liver. These processes occurs within a 10-min period. After 30 min, CI are excreted with bile, which coincides with the appearance of *de novo* synthesized 200 kD CP in the bile. This CP was characterized *in vivo* with the use of radiolabeled amino acids [2]. Presumably, this isoform is programmed by the longest (4100 nucleotides) forms of the CP mRNA. Serum CP (labeled by the polypeptide chain), which is programmed by the classic form of CP mRNA [8,9], is secreted into the circulation 90 min after the start of the synthesis [2], while CP labeled by CI is detected in peripheral blood after 4 h [7]. These findings show that before metabolic binding to CP in hepatocytes, CI are bound to a certain mediator, which may be the intracellular CP-like protein (50 kD) [4]. The synthesis of this CP-like protein is probably programmed by the shortest (1800 nucleotides) CP mRNA. It can be suggested that this CP transports CI from membrane-bound proteins to CP secreted by hepatocytes into the circulation and bile [2].

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