its fractions on DNA synthesis in these cells. It is noteworthy that in addition to compounds with chalone activity, the preparation contains compounds that stimulate DNA synthesis.

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EXPERIMENTAL GENETICS

Intracellular Ceruloplasmin-Like Protein of Mammals

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Pulse-chase experiments show that, in addition to the secreted molecular forms of ceruloplasmin, an intracellular ceruloplasmin-like protein is synthesized in rat hepatocytes. Radioimmunochemical assay demonstrates that a nonserum isoform of ceruloplasmin is bound to the Golgi membranes.

Key Words: *protein biosynthesis; copper metabolism; ceruloplasmin; molecular forms; intracellular localization*

A family of ceruloplasmin-like proteins play an important role in the transport, redistribution, and excretion of copper in the mammalian organism [6,8,10]. The least studied stage of copper metabolism is its intracellular transport to the sites where copper-containing enzyme-proteins are synthesized. In this connection we tried to discover a non-secretory, intracellular molecular form of ceruloplasmin (CP).

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MATERIALS AND METHODS

Biosynthesis and subsequent migration of immunoreactive, *de novo* synthesized CP were studied in pulse-chase experiments. Hundred-milligram aliquots of adult rat hepatic tissue, minced with scissors, were incubated under automatic stirring in 1 ml of Hanks' solution containing 10 mM glucose, 1%

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bovine serum albumin, 100 μCi [^{35}S]methionine, and 40 μM of an equimolar mixture of the remaining 19 unlabeled amino acids. Following a 15-min incubation at 37°C, a 500-fold excess of unlabeled methionine was added to each sample, and incubation was continued under the same conditions for 0, 15, 45, 75, and 105 min, respectively. After incubation was completed, the samples were immediately ice-chilled, and the incubation medium and tissue were separated by centrifugation for 10 min at 2000 g. The supernatant (incubation medium) was additionally purified by centrifugation (20 min, 23,000 g). The pellet was homogenized, and the total cell membrane fraction was prepared from the postnuclear supernatant. Immunoreactive CP was precipitated from both fractions by monovalent polyclonal antibodies to CP, as we described previously [7].

The Golgi membranes were isolated by flotation in a stepwise sucrose concentration gradient [5]. Proteins of the Golgi apparatus, disintegrated by sonication, were chemically iodine-radiolabeled with the use of chloramine T. Native immunoresponsive molecules of CP were identified by a reverse immunoblotting technique developed by us [4].

A confluent, fourth-passage CV-1 cell culture was used in the study. After the surface of a flask was completely covered, the culture medium was replaced with a DMEM medium without methionine, 50 μCi/ml [^{35}S]methionine was added, and incubation was performed for 4 h. The culture medium was clarified by centrifugation for 20 min at 23,000 g. The protein fraction was precipitated with a 20% solution of polyethyleneglycol-6000 (PEG) and dissolved in a minimum volume of physiological saline. The cells were harvested mechanically and lyzed with 1% Triton X-100. The postnuclear fraction was centrifuged for 20 min at 23,000 g and the cytosol (the supernatant) and the total cell membrane fraction (the pellet) were separated. Rocket immunophoresis was performed in 1% agarose gel in the presence of 0.5 % Triton X-100 and 4% PEG-6000. Electrophoresis under denaturing conditions was performed in polyacrylamide gel (PAAG) in the presence of sodium dodecylsulfate [9].

RESULTS

Intracellular migration of *de novo* synthesized CP was investigated in the pulse-chase experiments. Figure 1 shows that labeled CP did not appear in the incubation medium until after 30 min after the start of synthesis. We demonstrated previously that the mean time of synthesis of the polypeptide chain of the CP is approximately 10 min [1]. Some 20 min are required for intracellular post-translation maturation of the molecular form of CP, which is secreted by hepatocytes with the bile. Polar secretion of the serum form of labeled CP is delayed, beginning no sooner than 60 min after the start of its synthesis and ceasing within 90 min [3]. Thus, the time course of the *in vitro* secretion of CP (Fig. 1) completely reflects the overall process of the polar secretion of two molecular forms of CP which proceeds in the liver *in vivo*. The data presented in Fig. 1 also demonstrate that after secretion is terminated, *de novo* synthesized CP, which is yet to be secreted fully even 105 min after the start of the chase, is found in the membrane fraction of hepatocytes. These data are indicative of the synthesis of an intracellular CP-like protein which is identified by reverse immunoblotting as an individual electrophoretic form among the proteins bound to the total membrane fraction of hepatocytes [4]. As is seen from Fig. 3, *a*, along with the CP isoforms corresponding in electrophoretic mobility to CP from the serum and from the bile, a CP isoform with R_f=0.5

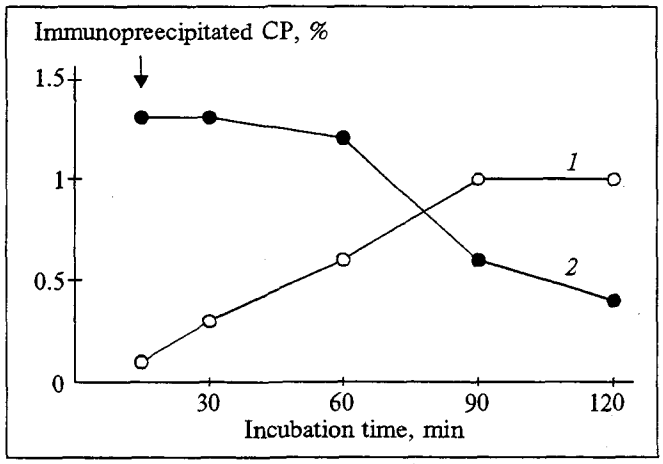

Fig. 1. Dynamics of distribution of *de novo* synthesized immunoresponsive CP between incubation medium (*1*) and membrane fraction of rat hepatocytes (*2*). Time of addition of unlabeled methionine shown by an arrow.

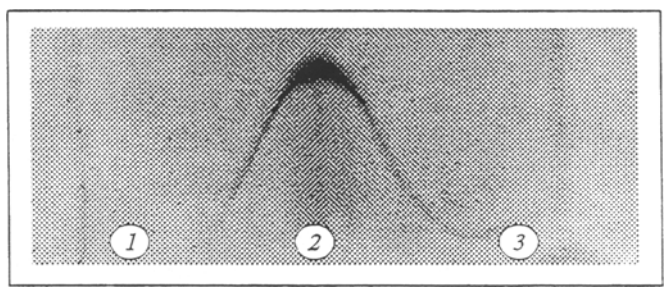

Fig. 2. Rocket immunoelectrophoresis of different fractions of CV cells against antibodies to CP. *1*) incubation medium; *2*) cell membrane fraction; *3*) cell nucleus fraction. One milliliter of 1% agarose gel contained 50 μg of rabbit immunoglobulins against human CP.

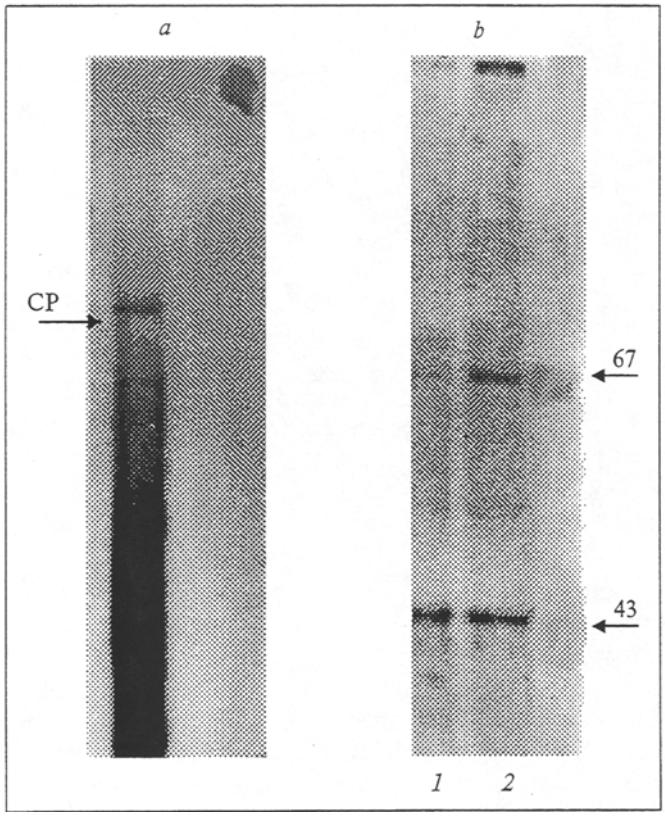

Fig. 3. Detection of molecular forms of CP in mammalian cells. *a*) CP isoforms from Golgi apparatus of rat liver. Iodinated cisternal proteins of Golgi membranes, electrophoretically separated in 7.5% PAAG under native conditions, were transferred by immunoblotting onto a nitrocellulose filter presaturated with antibodies to rat CP [4]. The filter was rinsed and exposed with an RT−1 X−ray film for 48 h. Position of rat serum CP shown by an arrow. *b*) distribution of *de novo* synthesized immunoreactive polypeptides of CP in CV−1 cells. *1*) [^{35}S]CP from cytosol; *2*) [^{35}S]CP from lyzed membrane fraction. Electrophoresis of immunoprecipitates was performed in 7.5% PAAG in the presence of sodium dodecylsulfate. Position of marker proteins (bovine serum albumin, 67 kD; and ovalbumin, 43 kD) shown by arrow.

is bound to the cell membranes. The liver is an unsuitable model for studying the biosynthesis of intracellular CP, since several isoforms of CP are simultaneously synthesized in hepatocytes. Synthesis of a nonsecretory form of CP has been shown to proceed in few organs including the kidney, which plays an important role in copper metabolism [6]. Therefore, the CV-1 cell strain initially obtained from the kidney of a grivet (*Cercopithecus aethiops*), whose CP exhibits the same antigenic properties as human CP, was chosen by us as a model in studies of the biosynthesis of intracellular CP-like protein [7]. Rocket immunoelectrophoresis demonstrates that the CV-1 cells synthesize intracellular immunoreactive CP (Fig. 2). Analysis of the molecular weight of [^{35}S]CP polypeptides derived from the CV-1 cells (Fig. 3, *b*) shows that: 1) the immunoresponsive CP polypeptides are not only bound to the membrane fraction, but are also encountered among the cytosol proteins; 2) the major polypeptides of the cytosol and membrane CP-like proteins have a similar molecular weight of approximately 48 kD. Our findings suggest that the independent intracellular molecular form of CP is present in different mammalian cells. This CP is the terminal site in the chain of structurally similar CP-like proteins which mediate the intercompartmental transport of extra- and intracellular copper ions. It seems likely that precisely this CP transfers the copper ions which enter the cell as the complexes of serum CP with CP receptor to the site of synthesis of intracellular copper proteins.

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