Transcriptional Regulation of Ceruloplasmin Gene Expression during Inflammation*

Jonathan D. Gitlin‡
From the Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110

Mixed sequence oligonucleotides were used to isolate a series of acute-phase human liver cDNA clones corresponding to the serum α2-globulin ceruloplasmin. These clones were characterized, sequenced, and used to analyze changes in hepatic ceruloplasmin mRNA content during inflammation. In all species examined, hepatic ceruloplasmin mRNA content increased approximately 6-10-fold over control values within 24 h following the induction of inflammation. The mechanisms leading to this increase in hepatic ceruloplasmin mRNA content were studied following turpentine-induced inflammation in Syrian hamsters. Nuclear run-on assays demonstrated an increase in the relative rate of transcription of the ceruloplasmin gene within 3 h following induction, reaching maximum values by 18 h. Hepatic ceruloplasmin mRNA content increased 2-fold within 12 h following induction, reached maximum values by 24 h, and returned to control within 72 h. In contrast, serum ceruloplasmin concentrations did not increase until 36 h, reached maximal levels by 120 h, and remained elevated for the course of the study. These data indicate that inflammation leads to a rapid increase in hepatic ceruloplasmin mRNA content. This increase is largely the result of increased ceruloplasmin gene transcription, but comparison of the relative rate of transcription and mRNA accumulation suggests that changes in ceruloplasmin mRNA turnover are also involved. In addition, mRNA content increased by the transcriptional and/or post-transcriptional mechanisms must account for the observed changes in serum ceruloplasmin concentration seen during inflammation.

Ceruloplasmin is a serum α2-glycoprotein which contains greater than 95% of plasma copper. The protein is synthesized in the liver as a single polypeptide chain of 1046 amino acids and secreted into the plasma with six atoms of copper bound per molecule (1). The complete amino acid sequence of ceruloplasmin has been determined (2) and cDNA clones have been isolated from human (3-6) and rat (7) liver libraries. The nucleotide-derived amino acid sequence data (3, 4) confirm the published amino acid sequence, although one report suggests the possibility of two ceruloplasmin moieties with heterogeneous carboxyl termini (4). The sequence of ceruloplasmin reveals a 3-fold internal repeat suggesting that gene duplication events may have been involved in the evolution of the current molecule (8). Sequence similarity has been noted to factors V and VIII of the coagulation cascade suggesting a common evolutionary origin for these proteins (9). In addition more limited similarity has been noted to plastocyanin (10), azurin (11), laccase (12), superoxide dismutase (13), and cytochrome oxidase (14), all presumably related to copper binding domains.

The functions of ceruloplasmin are not well defined but may include 1) copper transport and metabolism (15), 2) oxidase activity toward ferrous ions and aromatic amines (16), 3) antioxidant activity (17), 4) superoxide dismutase activity (18), and 5) tissue angiogenesis (19). Several of these proposed functions suggest that ceruloplasmin might be involved in host defenses, and indeed serum ceruloplasmin concentrations are increased during infection, inflammation, trauma, pregnancy, and certain malignant disorders (20). In addition, inherited disorders of copper metabolism in man and mice are associated with a marked decrease in serum ceruloplasmin concentration (21).

The molecular mechanisms regulating the changes in serum ceruloplasmin concentration have not been well characterized. Early studies suggested that estrogen and copper can increase ceruloplasmin biosynthesis in primary cultures of rat hepatocytes (22), and more recently interleukin-1 and other cytokines have been shown to have a similar effect on ceruloplasmin synthesis in human hepatoma-derived cell lines (23). A recent study in rats demonstrated that turpentine-induced inflammation leads to an increase in hepatic ceruloplasmin mRNA content in this species (7). In the present study, we have isolated and characterized hepatic cDNA clones corresponding to human ceruloplasmin and have used these to investigate the molecular mechanism of ceruloplasmin gene expression during inflammation.

EXPERIMENTAL PROCEDURES

Materials

Purchased chemicals and reagents including phenol were from Anachemica Chemicals, Montreal; guanidinium isothiocyanate was from Fluka, Switzerland; Seakem ME Agarose was from FMC, Rockland, ME; and nitrocellulose was from Schleicher & Schuell. All restriction enzymes were from Promega Biotech, Madison, WI, and used according to specifications. cDNA reagents were purchased from Amersham, and all nucleotides were from Du Pont-New England Nuclear. Syrian hamsters and Sprague-Dawley rats were purchased from the Charles River Breeding Labs, Boston, MA, and C57Bl/6J mice were purchased from the Jackson Laboratories, Bar Harbor, ME. All animals were maintained on normal diets in a germ-free environment. Human tissues were obtained under approved protocol from the patients at the Children's Hospital Medical Center, Boston, MA, and the Children's Hospital of Washington University Medical Center, St. Louis, MO.

Methods

Construction and Screening of cDNA Libraries—Mixed sequence oligonucleotides were synthesized on an Applied Biosystems Model 380A DNA synthesizer, purified on polyacrylamide gels, and labeled with [γ-32P]ATP using T4 polynucleotide kinase (24). Oligonucleotides

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corresponding to published amino acid sequence data for human ceruloplasmin (amino acids 604–610 and 802–809) (2) were used to screen an adult human acute-phase liver library (25) and two cDNA clones were isolated and characterized (pCP12 and pCP18) (Fig. 1).

To construct a second cDNA library, poly(A) RNA from acute-phase human liver was isolated on oligo(dT) cellulose columns (24) (Pharmacia type 7), and cDNA was synthesized according to the method of Gubler and Hoffman (26). The library was ligated into EcoRI cut pgt11, packaged, and plated on Escherichia coli Y1090, and screened with the original cDNA clones. Clones isolated from screening this library and two human hepatoma-derived cell line (HePG2) libraries (27, 28) were used to determine the sequence and structure of ceruloplasmin mRNA (Fig. 1). Nucleotide sequence analysis was performed by the dideoxy chain termination method of Sanger et al. (29).

Experimental Inflammation and RNA Isolation—Animals were injected with 0.5 cm³ of gum turpentine (0.5 cm³ of saline for controls) in the hindquarter and killed at various time points following injection. The skin was sterilized with 100% ethanol, the organs removed, rinsed in phosphate-buffered saline to remove blood, and frozen in liquid nitrogen. One g of frozen tissue was homogenized to powder, rinsed in phosphate-buffered saline to remove blood, and frozen in liquid nitrogen. One g of frozen tissue was homogenized (15 strokes in a type B Dounce homogenizer) in 10 ml of 0.32 M sucrose, 5 mM MgCl₂, 1 mM spermidine, 10 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 1 mM EDTA, 0.4% Nonidet P-40, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF). The homogenate was then filtered through four layers of sterile cheesecloth and diluted with an equal volume of 2.0 M sucrose, 5 mM MgCl₂, 10 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, and 0.1 mM PMSF (Buffer B). 3 ml of this solution was then layered over 1.6 ml of Buffer B and centrifuged for 1 h at 30,000 × g in a Beckman SW 50.1 rotor at 4 °C. The nuclear pellet was resuspended in 40% glycerol, 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 4 mM MnCl₂, 250 mM KCl, 5 mM dithiothreitol, and 0.1 mM PMSF. Nuclear RNA was stored in this buffer at −80 °C at a concentration of 10⁶ nuclei/ml.

Transcription reactions were carried out as described by McKnight and Baltimore (6), using 20 ml of nuclear RNA as a template. 3H-UTP (600 Ci/mmol) at 37 °C. The reaction mix was treated with proteinase K (Boehringer-Mannheim) (100 μg/ml) in 0.1 M Tris-HCl, pH 7.5, 10 mM EDTA, 0.15 M NaCl, and 2% SDS for 1 h at 37 °C. This was then diluted, extracted with phenol/chloroform three times, and ethanol-precipitated. Pelleted transcripts were washed in 70% ethanol and resuspended in RNase-free H₂O.

Hybridization of Transcripts—In addition to the ceruloplasmin clone pCP3, the plasmids used included pBR322 and pGEM-3 and cDNA insert containing plasmids for mouse albumin (33), human serum albumin (30), mouse chorion glycoprotein (34), and chicken actin (36), and human SAA (37). Control and insert containing plasmids were linearized by restriction endonuclease digestion, phenol-extracted, ethanol-precipitated, and resuspended in 0.2 M NH₄OH, 2.0 M NaCl at a concentration of 50 μg/ml. 5 μg of each plasmid solution was boiled for 2 min, cooled on ice, and blotted onto nitrocellulose using a Schleicher & Schuell filtration device. The nitrocellulose filters were then incubated for 2 h at 80 °C and air-dried. The filters were prehybridized for 36 h at 37 °C in the same solution containing 0.5 M NaCl, 20 mM NaH₂PO₄, 0.5 mM EDTA, pH 8.0. The liver was removed and minced into fine tissue in 20 ml of 0.32 M sucrose, 5 mM MgCl₂, 1 mM spermidine, 10 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 1 mM EDTA, 0.4% Nonidet P-40, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF).

To obtain cDNA clones corresponding to human ceruloplasmin, we screened a human acute-phase liver cDNA library with mixed sequence oligonucleotides as indicated in Fig. 1. The resulting clones (pCP12 and pCP18) were used to screen 500,000 recombinants in a second acute-phase library, and 27 clones were isolated and characterized. Three clones were eventually sequenced comprising a total of 3537 nucleotides encompassing the entire 1046 amino acids of the mature molecule, nine amino acids of a putative leader peptide, and 123 bases of 3' untranslated region. The nucleotide sequence data obtained agree with a previously published report (41). Measurement of o-dianisidine oxidase activity in serum was used in order to determine the sensitivity of the assay. The assay was carried out as described (42) with A₅₄₀ at 5, 15, and 30 min for all samples in triplicate and data quantitated as micromoles of substrate oxidized/ml/min.

RESULTS

To obtain cDNA clones corresponding to human ceruloplasmin, we screened a human acute-phase liver cDNA library with mixed sequence oligonucleotides as indicated in Fig. 1. The resulting clones (pCP12 and pCP18) were used to screen 500,000 recombinants in a second acute-phase library, and 27 clones were isolated and characterized. Three clones were eventually sequenced comprising a total of 3537 nucleotides encompassing the entire 1046 amino acids of the mature molecule, nine amino acids of a putative leader peptide, and 123 bases of 3' untranslated region. The nucleotide sequence data obtained agree with a previously published report (4), except for nucleotide differences in the region encoding amino acids 77–80 of the mature molecule. The sequence of clone pCP5 in this region is ATT ATC GGA GCT which differs from the reported ATT ATC AAA GCT changing amino acid 79 from lysine to glycine. Isolation and characterization of multiple clones corresponding to the 3' region of
ceruloplasmin from both liver and HepG2 cDNA libraries identified clones utilizing both polyadenylate sites previously reported (3, 4) but did not identify a reported second cDNA clone coding for four additional amino acids (3), suggesting that this species is in relative low abundance. No differences in the sequence of isolated HepG2 clones were found when compared to liver-derived clones (data not shown). Although each ceruloplasmin clone hybridized with RNA from several different species, only pCP3 corresponding to amino acids 560–786 (Fig. 1) was found to hybridize under conditions of high stringency identical to that used to analyze human RNA. This clone contained less than 20% overall homology with the published nucleotide sequence of factors V or VIII and did not cross-hybridize with other human mRNAs. For these reasons, we concluded that the RNA detected in various rodent species with pCP3 was indeed ceruloplasmin, and this clone was used in all subsequent experiments.

To determine the molecular basis for the increase in ceruloplasmin serum concentration seen during inflammation, we first analyzed ceruloplasmin mRNA size and content in total human liver RNA samples from a normal adult (Fig. 2, lane 1) and a healthy adult who sustained irreversible brain damage from trauma 26 h prior to death (Fig. 2, lane 2). Nucleic acid hybridization with human ceruloplasmin cDNA revealed that RNA from normal human liver contains two ceruloplasmin-specific mRNAs of 4.2 and 3.7 kilobases size. Following a traumatic insult there is an equivalent increase in the hepatic content of both transcripts without an accompanying change in transcript size. We used this same technique to analyze RNA from several different species, only pCP3 corresponding to amino acids 560–786 (Fig. 3A) was indeed ceruloplasmin, and this clone was used in all subsequent experiments.

FIG. 1. Cloning and sequencing of human ceruloplasmin. The oligonucleotide sequences used in cDNA library screening are shown in position within the 1046-amino acid protein structure. Redundancy of mixed sequences is indicated in parentheses. The clones isolated from this screening, pCp12 and pCp18, are shown in position above, and the three clones utilized for sequencing the 3537 nucleotides are illustrated at the top of the figure. Arrows indicate direction of sequence. @ represents clones sequenced following restriction enzyme subcloning, and O represents regions sequenced with specific oligonucleotide primers. The relationship of the sequence to the protein structure is shown in the rectangular box where filled regions represent 5' untranslated sequence and 5' leader peptide (amino acid 1 to 9), respectively. Restriction enzyme sites are shown as (E = XbaI, K = KpnI, + = EcoRI).

FIG. 2. RNA blot analysis of hepatic ceruloplasmin-specific mRNA. RNA was prepared and hybridized as described in text. Lanes 1 and 2 contain 15 μg of total human liver RNA from normal and acute-phase liver (approximately 26 h post-trauma). Lanes 3–8 contain 15 μg of total liver RNA from Syrian hamsters (3, 4), Sprague-Dawley rats (5, 6), and C3HeB/FeJ mice (7, 8) 24 h after the injection of saline (3, 5, 7) or turpentine (4, 6, 8). The blot was probed with pCP3 and exposed for 6 h as described.

FIG. 3. Analysis of hepatic ceruloplasmin specific mRNA in Syrian hamsters. A, RNA blot analysis of 15 μg of total liver RNA from male (lanes 1 and 2) or female (lanes 3 and 4) hamsters 24 h following intramuscular injection of saline (lanes 1 and 3) or turpentine (lanes 2 and 4). Blot was hybridized with human ceruloplasmin cDNA clone pCP3 and exposed for 2.5 h to highlight the difference in lanes 2 and 4. Each lane represents RNA from one animal. The experiment was repeated six different times with similar results. B, RNA blot analysis of 15 μg of total liver RNA from hamsters at indicated times following intramuscular injection of turpentine. Blot was hybridized and exposed as for A. Each lane represents RNA from one animal. This experiment was repeated five times (three males, two females) with identical results.

h following an intramuscular injection of turpentine and performed nucleic acid hybridization as above. In hamsters, rats, and mice (Fig. 2, lanes 4, 6, and 8), inflammation resulted in a 6–10-fold increase in the amount of detectable hepatic ceruloplasmin-specific mRNA. As in the human, these increases in specific ceruloplasmin mRNA content were not accompanied by changes in transcript size.

The above data suggested a similar molecular mechanism for the inflammatory regulation of ceruloplasmin gene expression in many different species, and thus we next sought to analyze this process in detail using a single species (Syrian hamsters). Previous studies had suggested sex-related differences in the regulation of ceruloplasmin serum concentrations. We, therefore, conducted experiments to compare the effects of turpentine-induced inflammation on hepatic ceruloplasmin-specific mRNA content in male and female hamsters. Analysis of hepatic RNA samples from male or female animals 24 h after the injection of saline (Fig. 3A, lanes 1 and 3) or turpentine (Fig. 3A, lanes 2 and 4) revealed that the turpentine-induced increase in hepatic ceruloplasmin mRNA content was consistently greater in female animals (Fig. 3A, lane 4). This difference was seen in rats and mice as well (data not shown), but in all cases was less than 2-fold (female
versus male) and not associated with changes in transcript size (Fig. 3A). We also studied hamster hepatic ceruloplasmin mRNA content and size as a function of time following the induction of inflammation with turpentine. As can be seen in Fig. 3B, hepatic ceruloplasmin mRNA content increased within 12 h following turpentine-induced inflammation reaching a maximum by 24 h and returning to base line within 48 h. These preliminary studies allowed us to conclude that inflammation results in an increase in hepatic ceruloplasmin-specific mRNA content without changes in the site of existing transcripts throughout the time course of the study.

To determine the basis for the increase in hepatic ceruloplasmin mRNA content following inflammation, we studied the relative rate of transcription of the ceruloplasmin gene in hepatic nuclei isolated from hamsters 24 h following the injection of saline or turpentine. 32P-Labeled nuclear transcripts were isolated from intact nuclei following in vitro transcription reactions, and equivalent amounts of transcript from inflamed and control nuclei were hybridized to specific cDNA clones immobilized on nitrocellulose. Studies using a range of concentrations of input plasmid DNA indicated that 5 μg represented DNA excess, assuring that signal intensity would vary only as the result of input transcript. Preliminary studies also indicated that both the initial rate of UMP incorporation and the amount of α-amanitin-sensitive transcription (67% of total) were similar in nuclei from control and turpentine-treated animals (data not shown). These findings were important because equivalent amounts of input transcript (counts/min) were utilized in all hybridization experiments. The size of run-on transcripts from these nuclei was not determined. Plasmids pBR322 and pGEM-3 served as negative controls because no transcripts would be expected to hybridize with these bacterial sequences. As seen in Fig. 4, the amount of transcript bound to the control plasmids was minimal and did not change during turpentine stimulation. In addition, a plasmid containing a clone for chicken actin served as a control for the effects of stimulation because the hepatic expression of this gene is not regulated by inflammatory stimuli. A basal level of actin gene transcription was detected in nuclei from control hamsters, and this was not increased by turpentine-induced inflammation (Fig. 4). A plasmid specific for human SAA (which cross-reacts with all rodent species) was used as a positive control in these studies, because the transcription of this gene has been shown to increase during the acute-phase response. As expected (Fig. 4), the level of SAA gene transcription was minimal in control animals, but increased at least 50-fold following turpentine-induced inflammation. When transcripts from these same nuclei were hybridized with the human ceruloplasmin cDNA, a basal level of gene transcription was detected (Fig. 2). The relative rate of ceruloplasmin gene transcription increased approximately 1.5–2-fold. Although basal transcription of albumin was easily detected (Fig. 4) using a cDNA probe specific for mouse albumin, no change in the relative rate of albumin gene transcription was seen following turpentine-induced inflammation, despite a 25% reduction in hepatic albumin mRNA levels at this time (data not shown).

Because we now had evidence that the relative rate of ceruloplasmin gene transcription is increased during inflammation, we next determined the kinetics of this process. Previous work on the transcription of acute-phase genes and our own preliminary kinetic studies (Fig. 3B) suggested that time points earlier than 24 h would be useful to examine. Hamsters were injected with either saline or turpentine and, at various time points between 0 and 46 h later, one animal from each group was killed and hepatic nuclei isolated. At the end of the experimental period, all the collected nuclei were used for run-on assays in a single experiment to control for variation in transcriptional activity related to assay conditions. The results of this analysis for one of three such studies are shown in Fig. 5. An increase in the relative rate of ceruloplasmin gene transcription was detected as early as 3 h following turpentine-induced inflammation. This relative rate of transcription increased 2–3-fold reaching a maximum at 16–18 h following induction, and then decreasing with similar kinetics such that the rate in inflamed animals was equivalent to that in controls by 36 h.

To quantitate hepatic ceruloplasmin-specific mRNA levels under these same experimental conditions, we isolated total RNA from equivalent amounts of liver at the time of nuclei

**FIG. 4.** Effect of inflammation on hepatic acute-phase gene transcription in the Syrian hamster. 24 h after the intramuscular injection of saline (control) or turpentine (inflammation (Inflamm.)), hepatic nuclei were isolated and in vitro run-on assays were performed as described under “Experimental Procedures.” 32P-Labeled nuclear run-on transcripts were isolated and equivalent counts/min (6 × 10⁶) were hybridized to immobilized cDNA insert containing plasmids (pBR322 + pGem-3, bacterial plasmids; pmAh, mouse albumin; pCP3, human ceruloplasmin; phsSAA1, human SAA; pActin, chicken actin). The source of plasmids is given in text. The immobilized plasmid dots were washed and treated as described under “Experimental Procedures,” and all were exposed to x-ray film for 16 h.

**FIG. 5.** Kinetics of ceruloplasmin gene transcription during inflammation. At each time point indicated, a pair of hamsters having received intramuscular saline or turpentine were killed, nuclei were isolated, in vitro nuclear run-on assays were carried out, as described under “Experimental Procedures,” and the nascent transcripts were hybridized to pBR322 and pCP3. The results were quantitated by liquid scintillation counting of individual dots and percent change from control calculated as counts/min pCP3 (turpentine)/counts/min pBR322 (turpentine) relative to counts/min pCP3 (saline)/counts/min pBR322 (saline) at each time point. This experiment was repeated a total of three times. There was less than 10% variation between the experiments in the calculated values for each time point.
isolation for run-on assays. The ceruloplasmin-specific mRNA was quantitated by dot blot analysis. Fig. 6 illustrates the data from one of three studies. Hepatic ceruloplasmin mRNA increases within 12 h of turpentine-induced inflammation, reaching a 4-6-fold increase by 24 h and returning to control values within 3 days. Although the rapid increases in hepatic ceruloplasmin-specific mRNA which we initially observed (Fig. 2) suggested to us the possibility of transcriptional gene regulation, we were surprised by our kinetic findings given previous data concerning serum ceruloplasmin concentration changes during inflammation. During inflammation ceruloplasmin serum levels are increased approximately 2-fold, and this increase is not usually detectable until 1-2 days following the inflammatory stimulus. Furthermore, maximum levels are reached around day 5, and serum concentrations remain elevated for several weeks past this point. We, therefore, quantitated serum ceruloplasmin concentrations during the course of our RNA analysis. These data are shown in Fig. 7 and confirm the previously reported serum ceruloplasmin concentration changes seen during inflammation in hamsters and other species (30). In these studies serum ceruloplasmin concentration was measured by assay of plasma oxidase activity. At several time points these values were confirmed by immunological methods. The serum ceruloplasmin concentration in control hamsters was 32 mg/dl. This value increased to 87 mg/dl at 48 h and remained elevated past 7 days of study. This analysis allowed us to conclude that functional changes in serum ceruloplasmin oxidase activity were not occurring during the time course of these studies.

**DISCUSSION**

The purpose of this study was to isolate cDNA clones corresponding to human ceruloplasmin, to characterize and sequence these clones, and to use them to elucidate the molecular mechanisms of ceruloplasmin gene expression during inflammation. Our sequence data agree with that reported with one exception, the two nucleotide change at amino acid 79 which results in a change from lysine to glycine at this position. It is possible that this difference represents a polymorphism. The amino acid sequence data reported on human ceruloplasmin from pooled plasma suggests that a Lys/Gly polymorphism exists at amino acid 79 (2). A two-nucleotide change resulting in such a polymorphism would be unusual but has been reported for hemoglobin variants (43). Such polymorphisms, occurring at the site of exon-intron boundaries, have recently been seen for human albumin. Confirmation of these results must await genomic sequence information.

This study demonstrates that inflammation results in an increase in the content of hepatic ceruloplasmin-specific mRNA within 12 h following the initial stimulus. The analysis of transcriptional rates reveals that an increase in ceruloplasmin gene transcription is the major factor regulating this increase in hepatic ceruloplasmin mRNA content during inflammation. This finding is consistent with recent observations on the regulation of expression of several acute-phase protein genes including SAA (44), CRP (45), and others (46, 47). However, comparison of the kinetics of ceruloplasmin gene transcription (Fig. 5) and hepatic mRNA accumulation (Fig. 6) indicates that mechanisms in addition to transcriptional regulation are responsible for the increase in hepatic ceruloplasmin mRNA content. Following an inflammatory stimulus, the relative rate of ceruloplasmin gene transcription reaches a maximum at 16 h and is declining between 16-24 h, a time when hepatic ceruloplasmin mRNA is still accumulating. A similar discordance in the kinetics of transcriptional rate and mRNA accumulation has been reported for other hepatic acute-phase proteins including SAA (44), α1-acid glycoprotein (48) and rat α2-macroglobulin (49). Mechanisms responsible for this delay in hepatic ceruloplasmin mRNA accumulation would include changes in the rate of processing or transport of nuclear transcripts or a change in the rate of ceruloplasmin mRNA degradation during the inflammatory course. Interestingly, these post-transcriptional mechanisms may be responsible for the consistently greater increase in hepatic ceruloplasmin mRNA content seen in female animals following inflammation (Fig. 3A), because no sex difference was detected in the analysis of transcriptional rates (data not shown). Estrogen may be implicated in this process because estrogen increases specific mRNA content through stabilization of selected transcripts (50).

Our results indicate that the serum concentration of ceruloplasmin is increasing (Fig. 7) at a time when hepatic mRNA

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*F. W. Putnam, personal communication.*
content is decreasing toward base line (Fig. 6) and that the serum concentration remains elevated long after hepatic mRNA content has returned to preinflammation values. This situation is not unique to the hamster since the kinetics of ceruloplasmin serum changes are similar to that reported in humans and other species following inflammation (20). Furthermore, the ceruloplasmin hepatic mRNA kinetics post-turpentine seen here are similar to those reported in a recent study in rats (7). Several possibilities for these findings must be considered. First, because the half-life of ceruloplasmin in these animals is approximately 2 days and plasma ceruloplasmin concentrations remain elevated for at least 2 weeks, these serum concentrations cannot result simply from an increase in hepatic synthesis in the first 48 h. However, such changes might be seen if the degradation of ceruloplasmin decreases during inflammation. This seems unlikely since the degradation of most plasma proteins increases slightly during inflammation and turnover data during the acute-phase response does not suggest any significant change in ceruloplasmin half-life (51). Secondly, a change in translational efficiency of ceruloplasmin mRNA might occur during inflammation such that ceruloplasmin mRNA content does not accurately reflect the amount of translatable message. While we have no data currently to support this, changes in the translational efficiency of specific mRNA during induction have been demonstrated for both ferritin (52) and C-reactive protein (53) suggesting the possibility of such a mechanism. A third possibility is that there is an increase in the efficiency of ceruloplasmin secretion during the course of inflammation. This has been demonstrated for C-reactive protein secretion by rabbit hepatocytes during an acute-phase response (54). Finally, it is possible that the serum concentration of ceruloplasmin reflects not only hepatic but also extrahepatic ceruloplasmin synthesis. Recent data have revealed extrahepatic ceruloplasmin gene expression in several organs in the rat (7), although the role of these tissues in inflammatory changes in ceruloplasmin production has not been characterized. We are currently testing each of these possibilities in our laboratory. Understanding these complexities of control of ceruloplasmin serum concentration are likely to be relevant not only to the acute-phase response but also to metabolic disorders of copper metabolism such as Wilson’s Disease where the mechanisms leading to decreased serum ceruloplasmin concentration remain unknown (55).

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