cDNA clones corresponding to rat ceruloplasmin were isolated from newborn rat lung and liver cDNA libraries and the nucleotide sequence was obtained. The derived amino acid sequence of rat ceruloplasmin is 93% homologous to the corresponding human sequence and contains a 19-amino acid leader peptide plus 1040 amino acids of mature protein. Southern blot analysis indicates that the ceruloplasmin gene exists as a single copy in the rat haploid genome. Using these cDNA clones in RNA blot analysis, a single 3.7-kilobase ceruloplasmin-specific transcript is detected in fetal rat liver and lung by day 15 of gestation. During fetal development the abundance of this transcript increases selectively in these two tissues and at birth is 60% of that found in the adult liver. Postnatally the temporal pattern of ceruloplasmin gene expression in lung and liver differs. Within the first 3 weeks postpartum ceruloplasmin mRNA content decreases in lung to undetectable levels, while that in the liver reaches adult levels. Primer extension reveals a single identical start site of ceruloplasmin gene transcription in lung and liver and biosynthetic studies indicate that each tissue synthesizes a ceruloplasmin protein which is qualitatively similar to that synthesized by adult liver. Ceruloplasmin mRNA is also detected in human fetal lung explant and a human lung adenocarcinoma cell line suggesting that a similar pattern of expression occurs in the developing human lung. These data indicate that lung is the predominant extrahepatic site of ceruloplasmin gene expression during fetal development and suggest that this protein may play a previously unappreciated role in lung development or pulmonary antioxidant defense.

Ceruloplasmin is a blue multi-copper oxidase which is found in the plasma of vertebrate species. The protein is synthesized in the liver as a single polypeptide chain and is secreted into the plasma with 6 atoms of copper bound per molecule (1). Ceruloplasmin circulates in the plasma as an α2-glycoprotein and contains greater than 90% of the copper found in this compartment. The complete amino acid sequence of human ceruloplasmin has been determined by protein sequence analysis (2) and confirmed by cDNA cloning (3, 4). This sequence data reveal that ceruloplasmin consists of three consecutive 350-residue homology units suggesting that the protein arose early in vertebrate evolution by duplication of a primordial gene encoding a copper oxidase (5). Ceruloplasmin contains three spectroscopically different copper centers (6) and has remarkable sequence similarity to other evolutionary diverse members of the multi-copper oxidase family including Neospora crassa lactase (7) and cucumber ascorbate oxidase (8). Although there is no detailed information on the sites of copper incorporation in ceruloplasmin, analysis of these sequence homologies suggests conserved domains which may be involved in copper binding. There are currently no data available on the primary structure of ceruloplasmin from species other than human.

Ceruloplasmin plays a role in a number of biological processes including tissue angiogenesis (9), copper transport (10), iron metabolism (11) and antioxidant defense (12, 13). Consistent with the role of each of these processes in host defense ceruloplasmin is an acute phase protein, and the serum concentration increases during inflammation, infection, and tissue repair (14). Hepatic copper content and serum ceruloplasmin concentrations are also altered during the perinatal and postpartum period. In the fetus reduced biliary copper excretion is associated with elevated liver copper and low serum ceruloplasmin activity. Following parturition the serum ceruloplasmin concentration rises coincident with a reduction in hepatic copper content (15). The mechanisms of these developmental changes have not been determined.

Although the liver is the major source of plasma proteins, extrahepatic synthesis of such proteins has been demonstrated (16, 17). Thus the function and regulation of ceruloplasmin must be considered in the context of extrahepatic tissue expression. Previous studies demonstrated ceruloplasmin synthesis in rat sertoli cells (18) and human synovial tissue (19). More recently extrahepatic ceruloplasmin gene expression was detected in adult rat testes and choroid plexus (20), fetal rat choroid plexus (21), and pregnant rat uterus (22). In the current study, we cloned and sequenced rat ceruloplasmin and examined the tissue-specific sites of gene expression during development. We report here on the primary structure of rat ceruloplasmin and that the lung is the predominant site of extrahepatic expression during development.

**EXPERIMENTAL PROCEDURES**

**Materials**

Chemicals and reagents used in this study included phenol from Anachemia Chemicals, Montreal; guanidinium isothiocyanate from Fluka, Switzerland; seakem GTG and ME-agarose from FMC Bio-products, MD; nitrocellulose from Schleicher & Schuell, and nylon (Hybond-N) from Amersham Corp. Additional chemicals were purchased from Sigma. All restriction enzymes were purchased from Promega Biotech (Madison, WI) and used according to the manufac-
turer's specifications. cDNA synthesis reagents were purchased from Invitrogen (San Diego, CA) and AMV reverse transcriptase from Life Sciences Inc. (St. Petersburg, FL). DNA and RNA modifying enzymes were purchased from Stratagene (San Diego, CA). 32P-Labeled nucleotides were purchased from Du Pont-New England Nuclear.

Adult male and female Sprague-Dawley and Wistar rats were purchased from Sprague-Dawley (Indianapolis, IN) and were maintained on normal diets. Adult rats were mated overnight and pregnancy was determined by the presence of a vaginal plug. The gestational age of all fetuses was calculated on this basis.

Methods

Construction and Screening of cDNA Libraries—cDNA libraries were constructed from newborn Sprague-Dawley rat lung and liver tissue (7). RNA was isolated on oligo(dT)-cellulose (22). For cDNA was synthesized as described (24). Hemiphosphorylated EcoRI linker adapters with an internal NotI site (Invitrogen) were ligated to the cDNA which was then sized by electrophoresis in a 1% agarose gel. cDNA fractions > 2.5 kb were recovered in DEAE paper, eluted, and ligated into the EcoRI site of pZapII (25). cDNA libraries were screened using a human ceruloplasmin cDNA clone (4) and positive clones were sequenced by the dyeoxy chain termination method using Sequenase (US Biochemicals, Cleveland, OH). The complete nucleotide sequence was determined (~85% from both strands) and sequence data including homology comparisons was analyzed using the Microgenie sequence analysis program (28).

Isolation of Genomic DNA and Southern Blotting—Genomic DNA was isolated from the spleen of an adult male Sprague-Dawley or Wistar rat (29) and from newborn lungs or livers (30) by the method described (31). Genomic DNA (50 μg) was digested with a series of restriction endonucleases and electrophoresed in 1% agarose gels. The gel was processed as described (23), transferred to nitrocellulose filters (34), and identical conditions were used for prehybridization, hybridization, and washing. An oligonucleotide corresponding to a region of the rat 28 S rRNA (33). RNA dot blots were prepared from total RNA samples applied to nitrocellulose filters (35). The probe used had an Az6/μAz8 > 1.8. RNA samples were denatured in formaldehyde gels. The size of specific mRNA transcripts was estimated during development, the developmental blots were reprobed using a cDNA for β-actin to ensure that equivalent amounts of RNA were present in each lane. Because cases RNA blots were reprobed with a cDNA for rat β-actin to ensure that equivalent amounts of RNA were present in each lane. Because the abundance of β-actin was also found to change in some tissues during development, the developmental blots were reprobed using a molar excess of an oligonucleotide complementary to rat 28 S RNA (54). RNA dot blots were prepared from total RNA samples applied to nitrocellulose filters (34) and identical conditions were used for prehybridization, hybridization, and washing. All dot blots were done in serial dilution (0.5-20 μg of RNA/sample) and in all cases the relative hybridization varied directly with the amount of input RNA. Following hybridization, some samples were rehybridized with a cDNA for rat β-actin to ensure that equivalent amounts of RNA were added at each time point. After autoradiography, individual dots were excised and quantitated by liquid scintillation counting. Plasmids containing cDNA inserts were used in these studies included a partial ceruloplasmin cDNA corresponding to 420-1063 bp (prCp1-4), a full-length cDNA for rat Cu/Zn/superoxide dismutase (35), and a full-length cDNA for rat β-actin (36).

Oligonucleotide Synthesis and Hybridization—Oligonucleotides (60-mers) corresponding to the published sequences for rat supranctant protein A (37), rat α-fetoprotein, and rat serum albumin (38) were synthesized on an Applied Biosystems oligonucleotide synthesizer and were subsequently labeled with [32P]dATP (30 μCi/μmol) using T4 polynucleotide kinase (23). RNA blots were hybridized with oligonucleotide probes at 55 °C in a solution containing 50% formamide, 6 × SSC, 5 × Denhardt's, 0.06 M Na3PO4, pH 6.8, 100 μg/ml single stranded DNA, and 0.05% NaPPi. Following 18 h of hybridization the blots were washed in 6 × SSC for 10 min at room temperature followed by washing in 50% formamide/6 × SSC for 2 h. A 26-mer oligonucleotide complementary to a region of the rat 28 S RNA (33) was synthesized and labeled as described above. This probe was then used in 10-fold molar excess for hybridization to RNA blots as described (39).

Primer Extension—Approximately 30,000 cpm of a 5' end-labeled oligonucleotide corresponding to the first 20 amino acids of the mature rat ceruloplasmin sequence was combined with 3 μg of poly(A) RNA (newborn lung or liver) in 10 ml of 0.05 M Tris-HCl, pH 8.3, 0.55 M KCl, and 1 mM EDTA. The samples were sealed in glass capillary tubes and incubated for 1 h at 60 °C (40). After allowing samples to cool to room temperature for 10 min, each sample was added to 0.05 M Tris-HCl, pH 8.3, 0.15 M KCl, 0.7 mM dNTP, 5 mM dithiothreitol, and 425 units/ml of AMV reverse transcriptase (Life Sciences). The transcriptase reaction was carried out for 2 h at 42 °C and the reactions were subsequently stopped with 0.3 M sodium acetate, 0.01 M Tris-HCl, pH 7.5, 1 mM EDTA. The primer extended products were precipitated with 10% trichloroacetic acid for 2 h, collected by centrifugation, and analyzed on a 6% polyacrylamide urea gel using known sequence reactions as size standards.

Cell and Organ Cultures—Human Hep G2, Hep 3B, CaCo, and HeLa cells were obtained as stock cultures from the American Type Culture Collection and maintained in culture according to specifications. NCI-H441-4 lung adenocarcinoma cells (SAP-1) were cultured as described (41). RNA was isolated from the cell lines as indicated above for whole tissue. Exemplar cultures of human fetal lung tissue were established and maintained as described (42).

Biosynthetic Labeling, Immunoprecipitation, and SDS-PAGE—Fresly removed newborn lung, brain, or liver tissue (1 g) was minced in methionine-free minimal essential media and then incubated at 37 °C in methionine-free media containing [35S]methionine (500 μCi/ml). Following a 2 h incubation the culture media was removed and tissue was lysed by freeze/thawing followed by solubilization of the lysate (43). Total protein synthesis was estimated by trichloroacetic precipitation of aliquots of tissue lysates and media (44). Both lysate and media were clarified by centrifugation at 4°C for 0.5 h at 10,000 ×g in 1% Triton, 1% SDS, 0.5% deoxycholic acid with excess antibody. Immune complexes were precipitated with formalin-fixed staphylococci-protein A, released by boiling in sample buffer, and analyzed at 7.5% SDS-PAGE under reducing conditions (45). 125I-Methylated molecular size markers were included on all gels and following electrophoresis gels were impregnated with 2.5-diphenyloxazole (EHNACE, Du Pont-New England Nuclear) and dried for fluorography on XAR-5 film. Antibodies used in this study included rabbit anti-rat ceruloplasmin (46), rabbit anti-human ceruloplasmin (19), and rabbit anti-human albumin and anti-factor B (Atlantic Antibodies).

RESULTS

Characterization of Rat Ceruloplasmin Primary Structure—The complete nucleotide sequence of rat ceruloplasmin including 15 bp of 5'-untranslated sequence, 19 amino acids of a leader peptide, 1040 amino acids of mature protein, and 512 base pairs of 3'-untranslated sequence is shown in Fig. 1. The nucleotide sequence from 70-3715 bp was obtained from newborn lung cDNA clones and the remaining sequence (Fig. 1, 1-69 bp) was deduced from the sequence of the rat ceruloplasmin gene.2 Comparison of this sequence to that of human ceruloplasmin reveals significant homology (nucleotide 84%; amino acid 93%) (Fig. 1). However, a region of 12 amino acids (475-486) in the human sequence diverges completely in the

1 The abbreviations used are: kb, kilobases; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; bp, base pair.

2 R. Fleming and J. D. Gillin, unpublished data.
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sequence indicates a conservation of the Cys-His-Lys Type I Cu²⁺ ligands (5) in the later two homology units (rat amino acids 674–679–684; amino acids 915–920–925). Six of seven potential N-linked oligosaccharide attachment sites in the human are conserved in the rat; one site (Asn-339) is not because the Asn-X-(Thr) sequence is changed to Asn-X-Pro. This same site is variably glycosylated in human ceruloplasmin accounting for the two isoforms detected in serum (5).

Rat Ceruloplasmin Gene Exists as a Single Copy—To estimate the copy number of the ceruloplasmin gene in the rat haploid genome, the rat ceruloplasmin cDNA clones were used to analyze rat genomic DNA by Southern blot hybridization. Hybridization of restriction endonuclease-digested genomic DNA with cRNA probes derived from various portions of the full-length cDNA revealed patterns which were characteristic of a single copy gene approximately 45 kb in length. An example of such a pattern using prCpl-4 is shown in Fig. 2.

The Ceruloplasmin Gene Is Expressed in the Rat Lung during Fetal Development—RNA blot analysis of samples from liver, lung, heart, brain, placenta, and GI tract of 20-day rat fetuses revealed a single 3.7-kb ceruloplasmin mRNA in liver and lung tissue (Fig. 3). Hybridization of the same RNA samples with a full-length cDNA for rat Cu/Zn/superoxide dismutase identified a single 0.7-kb transcript in each tissue. The abundance of Cu/Zn/superoxide dismutase mRNA in the tissues is identical to that previously reported (47) and thus this data supports the conclusion that equal amounts of total RNA were present in each lane. A developmental analysis of liver, lung, heart, brain, placenta, GI tract, kidney, spleen, rat including an apparent omission of 5 amino acids. This sequence divergence could have been the result of a cloning artifact or a tissue-specific difference since the cDNA was obtained from lung and the human sequence was obtained from liver (3, 4). To resolve this problem we prepared a second cDNA library from newborn rat liver and isolated and sequenced ceruloplasmin cDNA clones. The sequences of the lung and liver clones were identical (data not shown) indicating that the species divergence is real and not the result of tissue-specific differences in protein sequence. The amino acid
skeletal muscle, and adipose tissue from day 16 to birth revealed a single 3.7-kb ceruloplasmin transcript only in liver and lung tissue at each gestational age, while control hybridization with a 28 S rRNA probe indicated that equivalent amounts of RNA were present at all time points in each tissue (data not shown). Trace ceruloplasmin mRNA was also detected in embryonic yolk sac, maternal uterus (Fig. 4), and lactating mammary gland (data not shown). Hybridization of RNA blots with oligonucleotide probes corresponding to proteins known to be specific to lung (surfactant A protein) or liver (albumin and α-fetoprotein) confirmed the organ fidelity of isolated samples (data not shown). An identical pattern of liver and lung ceruloplasmin gene expression was also obtained using fetal and newborn Wistar rats (data not shown).

The Temporal Pattern of Ceruloplasmin Gene Expression Is Different in Lung and Liver—RNA blot analysis of liver and lung tissue at various stages of development with cDNA probes corresponding to the entire ceruloplasmin sequence indicated no qualitative changes in ceruloplasmin gene expression (Fig. 5). Because of inherent difficulties in quantitating mRNA abundance by this method (i.e. reprobing of blot in Fig. 5 with β-actin revealed more RNA in newborn liver samples), the abundance of ceruloplasmin RNA was quantitated by dot blot analysis of total RNA from lung and liver tissue as illustrated in Fig. 6. These studies indicate that the abundance of ceruloplasmin-specific mRNA is equivalent in lung and liver throughout gestation increasing at term to 50-60% of that found in adult liver. Postnatally ceruloplasmin gene expression in the lung decreases such that by 3-4 weeks ceruloplasmin mRNA in this tissue is <10% of that seen in adult liver. In contrast, hepatic ceruloplasmin mRNA, already >60% of adult levels at birth, continues to increase postnatally reaching 70% of adult levels by 3-4 weeks.

A Single Transcription Start Site Used in Lung and Liver—

![Fig. 4](image-url)  
**Fig. 4.** RNA blot analysis of ceruloplasmin RNA in extra embryonic tissue. A, 10 μg of RNA from term placenta, uterus at day 6 and day 20 of gestation hybridized with prCpl-4. B, same blot as in A hybridized with rat β-actin. C, 10 μg of RNA from adult rat liver, newborn lung, and term uterus hybridized with prCpl-4.

![Fig. 5](image-url)  
**Fig. 5.** RNA blot analysis of ceruloplasmin mRNA in liver and lung. 10 μg of total RNA from day 18 (fetal), newborn (day 1), and adult (>6 weeks) Sprague-Dawley liver and lung tissue hybridized with prCpl-4 as discussed in text. An identical pattern is seen using probes corresponding to all portions of the full-length cDNA sequence (data not shown).

![Fig. 6](image-url)  
**Fig. 6.** Kinetics of ceruloplasmin gene expression during development. At each developmental time point indicated total RNA was isolated from liver and lung tissue, immobilized on nitrocellulose, and hybridized with prCpl-4 as described in text. Quantitation was carried out by liquid scintillation counting using an equivalent amount of immobilized yeast 18S rRNA as a control for backgrounds at each time point. The figure shows the results of a single experiment which was repeated in triplicate with each sample in four separate experiments. There was less than 10% variation between triplicate samples in each experimental time point and less than 15% variation between different animals at the same time points. The RNA from individual fetuses was pooled in each experiment. The results are displayed as a percent of adult liver defined as 100% for this analysis. Development times are as indicated assuming a 21-22-day gestational period. NB, first postnatal day; YA, 3 weeks; A, adults >6 weeks old.

![Fig. 7](image-url)  
**Fig. 7.** Determination of the initiation sites for transcription of the ceruloplasmin gene in rat lung and liver. A synthetic oligonucleotide complimentary to 70-109 bp of the rat ceruloplasmin sequence was used as a primer in the reverse transcriptase reaction. Details of the primary extension analysis are as described in the text. Reactions were carried out using 3 μg of poly(A+) RNA from day 21 fetal lung or adult liver with 30,000 cpm of oligonucleotide (lung, liver) or adult liver with 300,000 cpm of oligonucleotide (liver-AP). Primary extended DNA bands are indicated by the open arrows. The lowest arrow indicates the size of the primer probe alone (40 bp). A single transcription start site is seen in newborn lung and liver tissue.

An oligonucleotide (40-mer) corresponding to 70-109 bp of rat ceruloplasmin was used in primer extension to characterize the start site of the ceruloplasmin mRNA in lung and liver tissue. Analysis of the primer-extended products from rat lung and liver revealed that both tissues use a single identical start site for ceruloplasmin gene transcription (Fig. 7). In addition, a second primer-extended product was also detected in adult rat liver hybridized with an increased amount of oligonucle-
otide primer (Fig. 7, lane 3). This second product is not consistently detected under these conditions and appears to be an artifact of incomplete sample denaturation. This band is not detected in lung tissue even with excess primer during the transcriptase reaction (data not shown). As expected, no primer-extended product was detected when yeast tRNA was used as a control (Fig. 7, lane 5). When analyzed with sequence reactions as size markers, the primer-extension data predict that the complete ceruloplasmin mRNA contains an additional 15 bp of 5'-untranslated sequence beyond the 5' end of the sequence data as shown (Fig. 1). This data is consistent with data from genomic cloning.

The Expression of the Ceruloplasmin Gene in Newborn Lung Tissue Is Associated with Production of Ceruloplasmin Protein—To determine if the expression of ceruloplasmin mRNA in fetal and newborn lung tissue results in the production of ceruloplasmin protein, we isolated liver, lung, and brain tissue from newborn rats and immunoprecipitated ceruloplasmin from media (extracellular) and tissue lysate (intracellular) following biosynthetic labeling with [35S]methionine. As can be seen in Fig. 8 both lung and liver tissue synthesize and secrete a 132-kDa protein consistent with the single chain size of ceruloplasmin. Ceruloplasmin was not immunoprecipitated from newborn brain, a tissue previously shown not to contain detectable ceruloplasmin mRNA. Ceruloplasmin immunoprecipitated from tissue lysates of both lung and liver is seen as 2 bands approximately 4 kDa different in molecular mass (Fig. 8, intracellular, lanes 1 and 2). This doublet reflects the intracellular addition and processing of carbohydrate and is eliminated by preincubation of tissue for 6 h in tunicamycin (data not shown). The single band seen in media reflects the mature secreted form of the protein consistent with observations in hepatocyte cultures. In the experiments shown in Fig. 8 total counts/min in lysate and media were measured by trichloroacetic precipitation and ceruloplasmin was immunoprecipitated from equal counts/min in each tissue. Similar results were obtained using fetal tissues from days 18 and 20 of gestation and in each case the immunoprecipitation of newly synthesized ceruloplasmin was completely blocked by preaddition of purified rat ceruloplasmin but not rat albumin (data not shown).

A Human Type II Cell Line and Human Fetal Lung Tissue Express the Ceruloplasmin Gene—Following our finding of ceruloplasmin gene expression in newborn rat lung, we examined human fetal lung tissue and human lung cell lines for ceruloplasmin gene expression. The left half of Fig. 9 illustrates an RNA blot with 10 μg of total RNA from human GI (CaCo), liver (Hep3B), placenta (BeWo), fibroblast (HeLa), and lung (SAP-1) cell lines. The blot was hybridized with a human ceruloplasmin cDNA, and both ceruloplasmin transcripts detected previously in human liver (4) were found in liver and lung cell lines. The right half of Fig. 9 depicts RNA from a human fetal lung explant culture (20-week gestational age). In explant culture at 20 weeks of gestation no ceruloplasmin mRNA is detected at day 0 (lane 6), but following 4 days in culture, a time associated with the appearance of differentiating alveolar epithelial cells, both ceruloplasmin transcripts are seen (lane 7). Analysis of two additional samples of human fetal lung tissue confirmed these observations (data not shown).

FIG. 9. RNA blot analysis of ceruloplasmin mRNA in human cell lines and fetal lung tissue. 10 μg of total RNA from human GI (CaCo), liver (Hep3B), choriocarcinoma (BeWo), fibroblast (HeLa), or lung (SAP-1) cells (lanes 1–5) was isolated and hybridized as described in text. 10 μg of RNA from human fetal lung tissue (20-week gestation) after 8 h (Day 0) or 4 days (Day 4) in explant culture and analyzed for human ceruloplasmin mRNA. Position of 28S and 18S as indicated. Blots were exposed to XAR film at −70 °C for 4 and 14 h, respectively.

D I S C U S S I O N

The derived amino acid sequence of rat ceruloplasmin reveals extensive homology (−92%) to human ceruloplasmin along the entire length of the proteins with the exception of a 12-amino acid region which occurs at the site of "autolytic" cleavage of the human protein (5). This sequence divergence may explain the absence of such cleavage products with rat ceruloplasmin and suggests that any proposed role for such fragments must take into account a relatively late evolutionary divergence. In addition to coding region homology, the first 45 bp of 3'-untranslated sequence of rat and human ceruloplasmin were found to be greater than 95% homologous (data not shown). Previous reports suggest that the 3'-untranslated regions of mRNAs encoding structurally homologous proteins can be markedly conserved among species (48). A search of primate and rodent data banks against this region indicated no sequence homology with other structurally unrelated proteins.

The high degree of sequence conservation between human and rat ceruloplasmin indicates selective pressure to maintain a tertiary structure essential (presumably) for copper-binding. The sites of copper ion binding in ceruloplasmin have not been clearly defined. Limited crystallographic data and sequence homology to other multi-copper oxidases suggests that a cluster of Cys-Met-His in both the second and third domains of human ceruloplasmin serve as ligands for the type I (blue) Cu+ (II) ions (5). Consistent with this concept these residues are entirely conserved in rat ceruloplasmin. More recently three-dimensional structural data for crystallized ascorbate...
binding in ceruloplasmin. Ceruloplasmin plays a role in this process either by directly affecting elastin metabolism or by donating copper to lysyl oxidase (54). It is possible that ceruloplasmin mRNA in lung tissue is equal to that of the liver during all stages of fetal development. The presence of ceruloplasmin mRNA in lung tissue is probably not simply the result of expression in differentiating fetal endodermal or epithelial tissue because kidney and GI tract (all portions) did not contain detectable ceruloplasmin mRNA. Pulmonary ceruloplasmin gene expression occurs prior to the alveolar phase of lung development in the rat (51). Interestingly either dietary copper deficiency or the administration of copper- and extrahepatic sites (65). 2) Active repression of ceruloplasmin gene expression involves a complex interplay of transcriptional and post-transcriptional events (63) and potential mechanisms would include: 1) tissue-specific selective loss (absolute or functional) of a trans-acting transcriptional activator. This might involve a transcriptional regulating protein present in both lung and liver and recognizing defined cis-acting sequences such as recently described for C/EBP protein (64). Alternatively such factors may recognize tissue-specific cis-acting sequences as reported for other proteins expressed in liver and extrahypertrophic sites (65). 2) Active repression of ceruloplasmin transcription in lung tissue mediated via common or unique cis-acting sequences as described for a-fetoprotein (66). 3) A change in cell-specific ceruloplasmin mRNA content (via either transcription or mRNA turnover) in lung tissue related to changes in cell type during development resulting in positional effects on cell-specific expression as described recently for glutamine synthetase (67) or a direct role of cell types expressing the gene. These mechanisms will be distinguishable by molecular cloning and analysis of the ceruloplasmin gene and by in situ hybridization of lung tissue.

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