4°C], and the final supernatant was adjusted to 0.3 M NaCl before being mixed with 40 μl of packed anti-(EE) protein G–Sepharose beads (per milliliter of supernatant) for 3 hours at 4°C. The beads were washed five times with lysis buffer containing 0.3 M NaCl, and then four times with the same solution without NP-40 and containing 0.15 M NaCl and 0.02% (w/v) Tween 20. Proteins were eluted in the final wash buffer supplemented with eluting peptide (150 μg ml^{-1}) (*19*) by washing three times with 1 volume (compared with packed beads) of buffer (each time the beads were incubated with the eluting solution for 20 min on ice). PAE cells were transfected by a similar protocol [30 μg of pCMV3-(EE)PKB(Ser^{473} to Asp) vector (*14*) or 3.0 μg of pCMV3 (myc)-PKB kinase vector or irrelevant DNA] except they were serum-starved for 12 hours before stimulation with PDGF (2 ng ml^{-1}) for 45 s; assays were conducted as described in (*6*). In parallel experiments to examine the effects of transient expression of PKB kinase on another protein kinase cascade, cells were cotransfected with (myc)–ERK-2. Anti-(myc)–directed immunoprecipitates were assayed as described by S. Cook *et al.* [*EMBO J.* **12**, 3475 (1993)].

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27. The assay mixture used to purify PKB kinase activities contained 1 μl of column fraction [γ-^{32}P]ATP (5 μCi, 1 μM final concentration); assay buffer [0.1 M KCl, 5 mM $MgCl_2$, 1 mM EGTA, 30 mM Hepes (pH 7.4)]; 2.5 μM (EE)-PKB [from stock PBS containing 1 mM dithiothreitol (DTT), 1 mM EGTA, and then mixed 1:1 (v/v) with glycerol; the kinase was purified from SF9 cells infected with clonal, recombinant baculovirus through its (EE)-tag and the eluting peptide was removed by gel filtration]; and phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine with or without D-D-S/A-PtdIns(3,4,5)P_3 [final concentrations in the assay mixture of 100, 100, 20, and 15 μM, respectively; the vesicles were prepared by sonicating a dry film of the lipids into 25 mM Hepes (pH 7.4) and were stored at 4°C for up to 3 days]. The assays were run for 12 min at 30°C, stopped by the addition of 400 μl of ice-cold 1% Triton X-100, 0.3 M NaCl, 10 mM EDTA, 1 mM sodium pyrophosphate, 10 mM β-glycerophosphate, 50 mM sodium fluoride, 1 mM EGTA, 0.01% azide, 25 mM Hepes (pH 7.4), and then 30 μl of anti-(EE) beads [4 μl of packed beads per assay mixture; protein G–Sepharose covalently crosslinked to a saturating quantity of anti-(EE) monoclonal antibody]. The tubes were mixed at 4°C for 25 min and then washed once with the above stop buffer and the ^{32}P content of each tube was quantitated with a Geiger counter. Column fractions were diluted so that a maximum of 40 to 45% of the total [γ-^{32}P]ATP was consumed in any assay. In assays designed to more accurately quantitate PKB kinase activity, the total volume was 12 μl with all additions scaled up in proportion; otherwise all steps were the same except the assay mixtures were incubated with anti-(EE) beads for 1 hour [resulting in a mean of 87% of (EE)-PKB being recovered on the anti-(EE) beads], they were washed three times, and their ^{32}P content was defined by liquid scintillation counting. The extent of PKB phosphorylation was estimated by measuring the ^{32}P incorporated into PKB and knowing the specific radioactivity of [γ-^{32}P]ATP in the assay mixture and the concentration of PKB (determined by analysis of both Coomassie-stained gels in the presence of standard amounts of bovine serum albumin and the maximum ^{32}P that can be incorporated into a known amount of PKB from [γ-^{32}P]ATP of defined specific radioactivity by an excess of PKB kinase).
28. A high-performance liquid chromatography–size exclusion column (SEC) was prepared with a Biosilect column (V_T 11.6 ml; Bio-Rad). Samples (35 to 45 μl) were loaded, the flow was 40 μl min^{-1}, and 80-μl fractions were collected. The SEC buffer contained 0.15 M NaCl, 20 mM Hepes (pH 7.4), 0.5 mM EGTA, 0.1 mM EDTA, 1% betaine, 0.03% Tween 20, 0.01% azide, 2 mM β-glycerophosphate, 1 mM DTT, and pepstatin A, leupeptin, aprotinin, and antipain (all 2 μg ml^{-1}).
29. [^{32}P]PtdIns(3,4,5)P_3 binding was assayed by protein immunoblotting (samples heated to 50°C with SDS sample buffer). The filter was incubated in phosphate-buffered saline (PBS) containing 1% NP-40, 1 mM EGTA, and 0.01% azide for 12 hours at 4°C. The filter was incubated for 30 min (room temperature) in the above solution, which also contained 0.1% cholate, phosphatidylserine and phosphatidylcholine at 50 μg ml^{-1}, 1 mM $MgCl_2$, and 1 mM DTT. [^{32}P]PtdIns(3,4,5)P_3 {prepared from recombinant p101/p120-PI3K, PtdIns(4,5)P_2, and [γ-^{32}P]ATP (*19*)} was sonicated into solution (10 μCi in 10 ml) and applied to a filter for 20 min at room temperature and then washed away with fresh solution (five times over 5 min) and finally with PBS containing 1% NP-40. The filter was air dried and autoradiographed.
30. To assay association of PKB and PKB kinases with lipid vesicles and the effects of different lipids on the phosphorylation of PKB, we prepared the lipid vesicles by sonicating dry lipid films into 0.2 M sucrose, 20 mM KCl, 20 mM Hepes (pH 7.4 at 30°C), 0.01% azide (to give 200 μM phosphatidylcholine, 150 μM phosphatidylserine, 20 μM phosphatidylethanolamine, 10 μM sphingomyelin) plus the indicated concentrations of inositol lipids in the final assay mixture. These were mixed with the relevant kinases in an assay buffer containing bovine serum albumin (1 mg ml^{-1}), 0.12 M NaCl, 1 mM EGTA, 0.2 mM calcium, 1.5 mM $MgCl_2$, 1 mM DTT, 0.01% azide, 5 mM KCl, 20 mM Hepes (pH 7.4 at 25°C), and about 50 nM free calcium (all final concentrations in the assay mixture) with or without [γ-^{32}P]ATP (1 μM final concentration) and (EE)-PKB (2.5 μM final concentration). If the assays were to estimate association of the kinases with the lipid vesicles, then after 4 min at 30°C the assay mixtures were centrifuged (200,000*g* for 20 min). Portions of the supernatants were removed for assays or immunoblotting. The pellets were rinsed rapidly with assay buffer, recentrifuged, and dissolved in SDS sample buffer. Phosphorylation of PKB or PKBs was quantitated as described above.
31. Single-letter abbreviations for amino acid residues are as follows: A, Ala;, C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
32. K.A. is an Australian National Health and Medical Research Council C. J. Martin/R. G. Menzies Fellow. P.T.H. is a BBSRC Senior Research Fellow. Work in the laboratories of C.B.R. and A.B.H. was supported by grants from the Wellcome Trust and the Biotechnology and Biological Sciences Research Council. DNA sequencing and oligonucleotide synthesis were superbly handled by the Microchemical Facility at the Babraham Institute.

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Role of Ceruloplasmin in Cellular Iron Uptake

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Individuals with hereditary ceruloplasmin (Cp) deficiency have profound iron accumulation in most tissues, which suggests that Cp is important for normal release of cellular iron. Here, in contrast to expectations, Cp was shown to increase iron uptake by HepG2 cells, increasing the apparent affinity for the substrate by three times. Consistent with its role in iron uptake, Cp synthesis was regulated by iron supply and was increased four- to fivefold after iron depletion. Unlike other iron controllers that are posttranscriptionally regulated, Cp synthesis was transcriptionally regulated. Thus, iron-deficient cells could increase Cp synthesis to maintain intracellular iron homeostasis, so that defects would lead to global accumulation of iron in tissues.

Iron is essential for many different biological processes, often functioning as a protein-bound redox element. However, iron in excess of cellular needs is extremely toxic, and its levels are precisely regulated (*1*). Defective regulation due to hereditary hemochromatosis or secondary iron overload leads to hepatic iron excess and injury, most likely due to iron-stimulated free radical reactions (*2*). Alterations of iron pools are implicated in neurodegenerative disease, aging, microbial infection, atherosclerosis, and cancer (*3*). The balance required to maintain appropriate intracellular iron concentration has led to the utilization of multiple mechanisms that regulate, primarily at the posttranscriptional level, the synthesis of iron transport and storage proteins (*4*).

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Ceruloplasmin (Cp) is a 132-kD monomeric copper oxidase implicated in iron metabolism because of its catalytic oxidation of Fe^{2+} to Fe^{3+} (ferroxidase activity) (*5*). Also, copper-deficient swine develop anemia that is overcome by Cp injection (*6–8*). Cp ferroxidase activity accelerates iron incorporation into apo-transferrin (*9, 10*), which may cause a negative concentration gradient of iron and cellular iron release. Patients with aceruloplasminemia have hemosiderosis characterized by low serum iron, high serum ferritin, massive iron deposition in multiple organs, and neurological deficit and diabetes (*11–13*), further implicating Cp in release of iron from tissue (*14–16*). In addition, multicopper oxidases homologous to Cp [fet3 in *Saccharomyces cerevisiae* (*14, 17, 18*) and fio1 in *S. pombe* (*19*)] facilitate high-affinity iron uptake by yeast. Thus, the yeast oxidases and Cp may promote iron fluxes but in opposite orientations with respect to the cell surface (*15*).

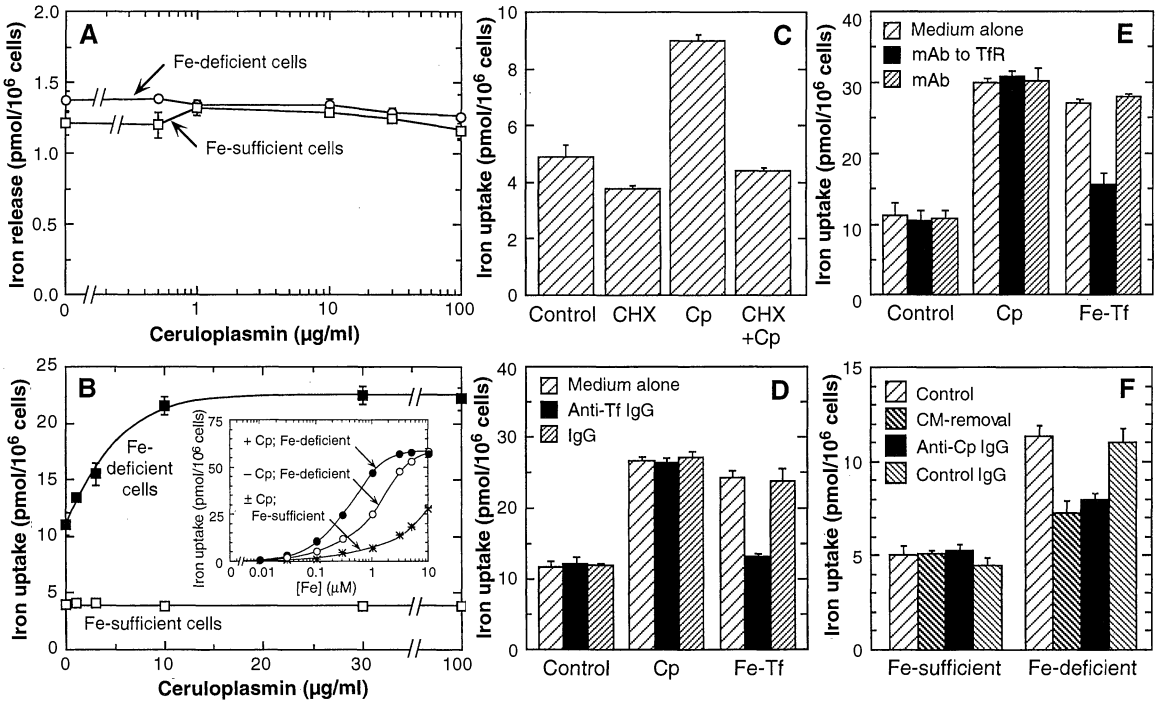

Fig. 1. Effect of Cp on iron flux in HepG2 cells. (**A**) Iron release (*30*) from confluent HepG2 cultures maintained in iron-sufficient Dulbecco's modified Eagle's medium–Ham's F12 (DME/F12) medium (□) or iron-depleted by treatment with 1 mM BP for 12 hours (○). (**B**) ^{55}Fe-NTA uptake (*31*) by HepG2 cells made iron-deficient by incubation for 16 hours with 1 mM BP (■) or maintained in iron-sufficient DME/F12 medium (□). Inset: Iron-deficient HepG2 cells were incubated with 0.01 to 10 μM ^{55}Fe-NTA and with Cp (30 μg/ml) (●) or medium alone (○). Likewise, iron-sufficient cells were incubated with (+) or without (×) Cp. (**C**) HepG2 cells were pretreated with cycloheximide (CHX; 10 μg/ml) for 1 hour and then made iron-deficient by BP treatment for 12 hours. Iron uptake was measured in the presence of purified human Cp (30 μg/ml). (**D**) Cp or transferrin (Tf) loaded with ^{55}Fe (*32*) was preincubated with rabbit anti-human IgG (1:1000; Accurate, Westbury, New York; black bars) or control IgG (1:1000; Accurate; dense striped bars) for 45 min and was added to cells pretreated with the same antibodies. ^{55}Fe-NTA uptake by iron-deficient HepG2 cells incubated with medium (control) or with Cp (100 μg/ml), and ^{55}Fe-transferrin (30 μg/ml) uptake, were measured after 15 min. (**E**) Iron-deficient HepG2 cells were preincubated for 60 min in medium alone (light striped bars), with monoclonal antibody (mAb) to the transferrin receptor (TfR) ?(2 μg/ml; Zymed, South San Francisco, California; black bars), or with an irrelevant antibody (2 μg/ml; dense striped bars). Iron uptake was measured after 15 min. (**F**) Iron-deficient (right) or iron-sufficient (left) HepG2 cells were allowed to condition their medium for 4 hours (open striped bars). In some wells, the conditioned medium was replaced after 4 hours with fresh medium before addition of ^{55}Fe (dense striped bars). In other wells, rabbit polyclonal anti–human Cp IgG (1:500; black bars) or a control IgG (1:500; light striped bars) was added to the conditioned medium 1 hour before the completion of the 4-hour incubation and ^{55}Fe uptake was measured.

The present studies were initiated to determine the role of Cp in cellular iron efflux, but activation of iron uptake was found.

The effect of Cp on iron transport was examined in HepG2 cells, a human hepatocellular carcinoma line exhibiting transferrin-independent and -dependent iron uptake (*20*). Iron release was measured from HepG2 cells preloaded with iron by incubation with ^{55}Fe-nitrilotriacetic acid (NTA). Cp did not significantly alter iron release by either iron-sufficient cells or by cells made iron-deficient by incubation with the Fe^{2+} chelator bathophenanthroline disulfonate (BP) (Fig. 1A). In view of these negative results and the known role of the Cp homolog fet3 in iron uptake by *S. cerevisiae*, we tested whether Cp stimulated iron uptake rather than release. Cp did not alter iron uptake in untreated, iron-sufficient HepG2 cells, but it approximately doubled uptake by iron-deficient cells (Fig. 1B). A half-maximal increase in iron uptake was observed at 5 μg of Cp per milliliter and a maximal increase was observed at 10 to 30 μg/ml, which are concentrations well below the normal range of 210 to 450 μg/ml in healthy adult human plasma. This observation may account for the apparently normal iron metabolism in patients with Wilson's disease who have lower than normal levels of plasma Cp and for iron deficiency in patients with levels less than 5% of normal (*21*). The increase in iron uptake by Cp was rapid, was not accompanied by an observable lag, and was linear for at least 40 min (*22*). Cp decreased the apparent Michaelis constant of iron uptake from about 1.2 μM to about 0.4 μM iron without altering maximal uptake (Fig. 1B, insert).

The inability of iron-sufficient cells to accumulate iron, even in the presence of Cp, suggested that a cellular component induced (or activated) by iron deficiency was required. Pretreatment of cells with cycloheximide prevented stimulation by Cp, which showed that a newly synthesized cellular protein or proteins are needed (Fig. 1C). One possible mechanism is that transferrin secreted by HepG2 cells (*23*) is iron-loaded through Cp ferroxidase activity (*10*) and carries iron into the cell via the transferrin receptor, which is induced by iron deficiency (*4*). Three experiments were done to show that Cp-mediated iron uptake did not use the transferrin pathway. Antibody to transferrin did not decrease Cp-mediated iron uptake by HepG2 cells; in a positive control, the antibody inhibited uptake of exogenous ^{55}Fe-transferrin (Fig. 1D). Similar results were obtained with a monoclonal antibody that blocked transferrin receptor endocytosis (*24*) (Fig. 1E). Finally, Cp enhanced iron uptake by iron-deprived K562 cells (*22*) that do not produce transferrin (*25*). Thus, the observed Cp-mediated iron uptake was transferrin-independent. Alternatively, the need for a newly synthesized cellular component is consistent with use of an inducible iron transporter analogous to ftr1p of *S. cerevisiae* (*26*).

Because HepG2 cells secrete Cp constitutively at a high rate (*27*), the role of endogenously produced Cp in iron uptake was examined. HepG2 cells were allowed to condition their medium for 4 hours to permit extracellular accumulation of Cp. Iron uptake by iron-depleted cells was two times more than uptake by iron-sufficient cells (Fig. 1F). Removal of the conditioned medium before measurement of iron uptake inhibited about 60 to 70% of the increase in iron-deficient cells, which suggests a role for a stable secreted factor. Addition of polyclonal rabbit anti-human Cp immunoglobulin G (IgG) (but not purified IgG)

to the conditioned medium decreased iron uptake almost to the level seen when the conditioned medium was removed, which shows that cell-derived Cp was responsible for most of the stimulatory activity secreted by iron-deficient HepG2 cells. Thus, iron uptake by HepG2 cells was mediated by cell-derived Cp in cooperation with a cell component induced by iron deficiency.

We investigated whether the synthesis of Cp, like that of other iron controllers (*4*), was regulated by cellular iron content. Iron depletion of HepG2 and Hep3B (*22*) cells by treatment with ferrous (BP, Fig. 2A) or ferric [desferrioxamine (DF), Fig. 2B] ion chelators increased Cp secretion four- to fivefold, according to immunoblot analysis using antibody to human Cp. Incubation of HepG2 cells with the copper chelator bathocuproine was completely ineffective (Fig. 2C), showing ion specificity. Going in the other direction, incubation of HepG2 cells with $FeCl_3$ decreased Cp synthesis by up to 50% as compared with that of control cells (Fig. 2D). The direction of these regulatory processes is consistent with a role for Cp in iron uptake and maintenance of cellular iron homeostasis. Based on the mechanisms known to regulate iron-dependent synthesis of the transferrin receptor and ferritin, Cp synthesis may be expected to be regulated by analogous posttranscriptional mechanisms (*4*). After a 4-hour lag period, treatment of HepG2 cells with iron chelators increased the steady-state level of the two major Cp transcripts two- and fourfold after 8 and 16 hours, respectively (Fig. 3A). The lag suggested that new synthesis of cellular proteins was required; this was verified by the ability of the protein synthesis inhibitor cycloheximide to completely block Cp mRNA induction by iron chelators (Fig. 3B). The possibility that iron depletion resulted in increased Cp mRNA stability, analogous to the regulation of the transferrin receptor (*4*), was examined. Cp mRNA accumulation was induced by iron chelation, actinomycin D was added to block new transcription, and the time course of mRNA decay was determined. Cp mRNA was extremely stable, exhibiting a decay of less than 15% during 10 hours (Fig. 4, A and D). Neither BP (Fig. 4, B and D) nor DF (Fig. 4, C and D) significantly enhanced Cp mRNA stability, which suggests that the effect was transcriptional. A nuclear run-on assay indicated at least a fivefold increase in the rate of Cp transcription by both chelators (Fig. 4E).

The finding that Cp stimulates cellular iron influx was surprising in the context of recent studies of aceruloplasminemia patients. One possible explanation is that defective Cp-mediated iron uptake by the liver results in plasma iron accumulation [possibly in low-molecular-weight iron complexes, as in hereditary (*28*) and secondary (*29*) hemochromatosis] to a level that drives nonspecific uptake by most tissues. The stimulation of iron uptake by Cp in hepatic cells is consistent with the uptake system described in *S. cerevisiae*

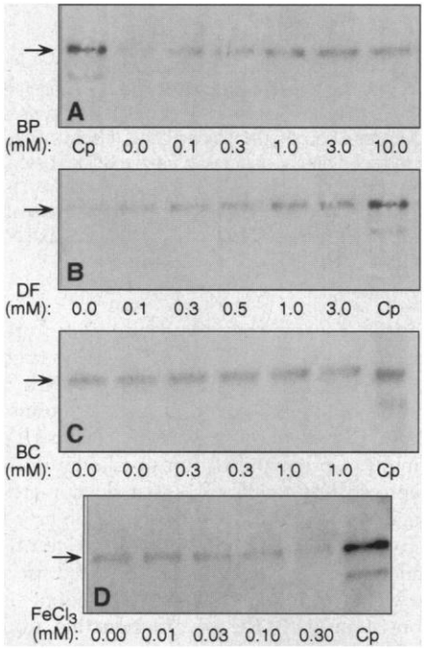

Fig. 2. Regulation of Cp synthesis by iron depletion. Confluent HepG2 cells in six-well tissue culture clusters were treated with (**A**) BP, (**B**) DF, (**C**) bathocuproinedi sulfonate (BC), and (**D**) $FeCl_3$ for 16 hours in serum-free DMEM/F12 medium. Aliquots of conditioned media [50 μl for (A) and (B), 100 μl for (C) and (D)] were subjected to 7% SDS–polyacrylamide gel electrophoresis and transferred onto Immobilon-P membranes (Millipore, Bedford, Massachusetts). The membranes were incubated with rabbit anti–human Cp IgG (1:20,000; Accurate) as primary antibody, then with peroxidase-conjugated secondary antibody (1:10,000; Boehringer Mannheim, Indianapolis, Indiana), and developed with the use of ECL (Amersham). Purified human Cp (Cp; 30 ng) was used as standard; the intact 132-kD protein is indicated by an arrow.

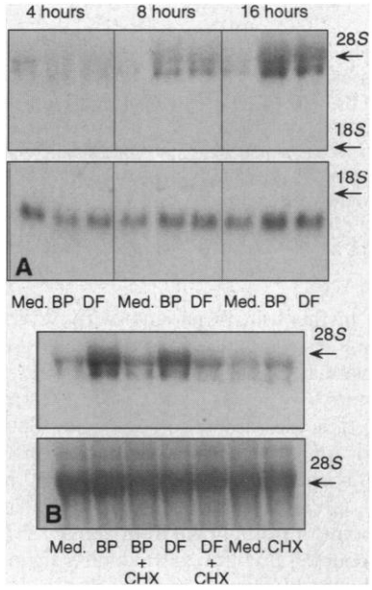

Fig. 3. Regulation of Cp mRNA steady-state levels by iron chelators. (**A**) Confluent HepG2 cells (5×10^6 cells) were treated for 4, 8, or 16 hours with 1 mM BP or DF or with medium (Med.) alone. Total RNA was harvested with RNAzol, and equal amounts of RNA (20 μg) were fractionated by formaldehyde agarose gel electrophoresis (1%) and then blotted on to nylon membranes. The blot was probed first with a 646–base pair fragment (Bst X1 and Bam H1) of a Cp cDNA labeled by random priming (top). Subsequently, the blot was stripped and reprobed with a random prime-labeled human GAPDH cDNA probe as a control (bottom). (**B**) Confluent HepG2 cells were pretreated for 1 hour with CHX (10 μg/ml), then with 1 mM BP or DF for an additional 14 hours. Total RNA was collected and subjected to RNA blot analysis; the blot was probed with a human Cp cDNA (top), and equal RNA loading was shown by the 28*S* ribosomal RNA bands (bottom).

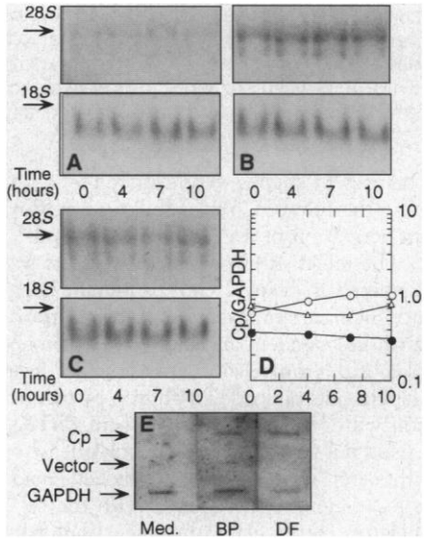

Fig. 4. Transcriptional regulation of Cp in iron-deficient HepG2 cells. Confluent HepG2 cultures were treated for 10 hours with medium alone (**A**), with 1 mM BP (**B**), or with 1 mM DF (**C**). Actinomycin D (5 μg/ml) was added, and the cells were harvested after 4, 7, and 10 hours. Total cellular RNA was isolated, and 20 μg of RNA were fractionated on an agarose gel, transferred to nylon membrane, and hybridized with human Cp cDNA (top) and with a human GAPDH cDNA probe (bottom). (**D**) Cp mRNA was normalized to GAPDH after quantitation by densitometry. Control cells (●) and cells treated with BP (○) or with DF (△) are shown. (**E**) Confluent HepG2 cells were treated with 1 mM BP or DF for 8 hours, and Cp transcription was measured by nuclear run-on assay (*33*).

and *S. pombe*. Those results are consistent with a model in which iron depletion of normal hepatocytes causes increased transcription of Cp that interacts with an iron transporter (also up-regulated by iron depletion), resulting in increased iron influx. The iron depletion requirement for Cp-stimulated Fe uptake, and the inhibition by cycloheximide, are consistent with a requirement for an inducible iron transporter. Taken together, the experiments in yeast and hepatic cells demonstrate a remarkable evolutionary conservation of the mechanisms that underlie the pathway controlling eukaryotic iron metabolism by copper. However, there are noteworthy differences: in yeast, the Cp homolog is a membrane protein that is co-transported to the cell surface and is in continuous contact with the iron transporter, whereas in mammalian tissues Cp is a secreted protein, and any interaction with a transporter is likely to be transient. This difference may be related to paracrine requirements for Cp in multicellular organisms.

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30. For measurement of iron release, cells were preloaded with iron by incubation with 100 μM ^{55}Fe-NTA for 4 hours. The cells were washed with saline-EDTA and incubated with Cp for 30 min in RPMI medium; the amount of ^{55}Fe released into the conditioned medium was measured by liquid scintillation counting.
31. For measurement of iron uptake, cells were washed with phosphate-buffered saline and then with iron-free RPMI medium. Cells were incubated with purified human Cp (Calbiochem, San Diego, CA) in the presence of 0.5 μM ^{55}Fe-NTA and 1 mM ascorbic acid in RPMI medium for 15 min at 25°C. The cultures were washed with saline-EDTA, and the cells were harvested with NaOH; after neutralization, cellular ^{55}Fe was measured by liquid scintillation counting.
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33. Isolated nuclei from 5 × 10^7 HepG2 cells were incubated with [α-^{32}P]uridine triphosphate (100 μCi, 3000 mCi/mmol). The purified reaction products were hybridized to linearized plasmid DNAs [Cp, 15 μg of pcDNA3-Cp; vector control, 15 μg of pcDNA3; and 1 μg of pcDNA3–glyceraldehyde phosphate dehydrogenase (GAPDH)] immobilized onto a nylon membrane.

34. We are grateful to J. Gitlin (Washington University) for the full-length Cp cDNA and for helpful discussions. This work was supported by NIH grants HL29582 and HL52692 (P.L.F.) and by a Fellowship Award from the American Heart Association, Northeast Ohio Affiliate (C.K.M.).

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Molecular Analysis of Cellulose Biosynthesis in *Arabidopsis*

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Cellulose, an abundant, crystalline polysaccharide, is central to plant morphogenesis and to many industries. Chemical and ultrastructural analyses together with map-based cloning indicate that the *RSW1* locus of *Arabidopsis* encodes the catalytic subunit of cellulose synthase. The cloned gene complements the *rsw1* mutant whose temperature-sensitive allele is changed in one amino acid. The mutant allele causes a specific reduction in cellulose synthesis, accumulation of noncrystalline β-1,4-glucan, disassembly of cellulose synthase, and widespread morphological abnormalities. Microfibril crystallization may require proper assembly of the *RSW1* gene product into synthase complexes whereas glucan biosynthesis per se does not.

Cellulose, a crystalline β-1,4-glucan, is the world's most abundant biopolymer. Its biomass makes it a global carbon sink and renewable energy source, and its crystallinity provides mechanical properties central to plant morphogenesis and the fiber industries. The mechanisms that plants use in synthesis have not yielded to biochemistry or cloning by hybridization to genes encoding prokaryotic cellulose synthases (*1*). By combining chemical and ultrastructural analyses with map-based cloning, we show that the *Arabidopsis RSW1* locus encodes a glycosyl transferase that complements the *rsw1* mutant (*2*). The temperature-sensitive *rsw1* allele disassembles cellulose synthase complexes in the plasma membrane ("rosettes"), alters cellulose crystallinity, and disrupts morphogenesis. The gene product, which is closely related to the putative cellulose synthase catalytic subunit from cotton fibers (*3*), can therefore be used to manipulate the production and physical properties of cellulose, while the mutant links plant morphogenesis and cellulose production.

Mutants impaired in cellulose production were selected with the use of a radial swelling phenotype (*rsw*), which mimics responses of wild-type roots to cellulose synthesis inhibitors such as dichlorobenzoni-

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