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# Influence of altered transcription on the translational control of human ferritin expression

**(iron metabolism/gene regulation)**

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ABSTRACT **In this paper, we examine the response of a translational regulatory mechanism when changes in mRNA levels are induced. The gene that encodes the human ferritin heavy chain has been transfected into mouse fibroblasts. Stable transformants that express the human ferritin heavy chain have been isolated. This protein assembles into ferritin polymers and can co-assemble with host mouse ferritin. Biosynthetic rates of the expressed human ferritin varied over a wide range in response to perturbations in iron supply, but total and cytoplasmic messenger RNA levels remained unchanged. When changes in ferritin mRNA levels were induced by treatment with sodium butyrate, proportional changes in the biosynthetic rates of ferritin were observed, but the capacity for modulating biosynthesis in response to alterations in iron availability was preserved. These findings suggest that the final protein biosynthetic rate of a translationally regulated gene depends on both translational regulatory signals and underlying transcription rates.**

Ferritin is the major intracellular repository of iron. It is a highly conserved heteropolymer composed of 24 assembled subunits that are the products of the ferritin heavy (H)-chain and the ferritin light (L)-chain genes. It serves to sequester and thereby detoxify intracellular iron, which is not otherwise utilized in cellular metabolism (1). Biosynthetic rates of ferritin and cellular ferritin levels are regulated by changes in iron availability (2). Regulation of the level of ferritin in the lining cells of the intestinal mucosa plays a critical role in the controlled absorption of dietary iron and is likely to be abnormal in the common genetic disease hereditary hemochromatosis (3). The regulated uptake and availability of iron is intimately tied to cell proliferation. Thus, understanding the regulation of ferritin remains a central problem in cellular metabolism. It has long been known that iron enhances the biosynthesis of ferritin in the absence of alterations in either transcription or total ferritin mRNA (4–7). The recent cloning of the cDNA and genes for ferritin (8–12) now makes it possible to study in detail the mechanism of this regulation. Here we report the expression of the human ferritin H chain in murine cells. The human protein assembles into spheres and co-assembles with murine H and L chains, and its expression is regulated by changes in iron availability without discernible changes in ferritin mRNA levels. However, when cells are induced to increase levels of human ferritin mRNA by treatment with sodium butyrate, protein biosynthetic rates increase proportionally. Regulation of ferritin biosynthesis is retained over a wide range of changes in iron availability when mRNA levels are increased, but increased mRNA levels are correlated with an upward shift in the range of biosynthetic rates achieved by manipulations of iron delivery.

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## MATERIALS AND METHODS

**Expression, Assembly, and Regulation of Protein Biosynthesis.** Stable transformants that express human H-chain mRNA were prepared as previously described (12). Cells were biosynthetically labeled with a 2-hr pulse of 50 μCi of [$^{35}$S]methionine per ml and were lysed in 300 mM NaCl/50 mM Tris·HCl, pH 7.4/1% Triton X-100 containing protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride/10 μg of leupeptin per ml). Trichloroacetic acid-precipitable protein ($1.5 \times 10^7$ cpm) was immunoprecipitated with either a saturating amount of a polyclonal anti-ferritin IgG, which recognizes both mouse and human ferritins (Boehringer Mannheim), or a saturating amount of a monoclonal antibody specific for human H chain (courtesy of Paolo Arosio, University of Milan, Milan, Italy) (13). After 60 min on ice, 100 μl of a 50% (vol/vol) suspension of protein A-Sepharose in isotonic phosphate-buffered saline was added to each sample, and the mixture was tumbled overnight. The resin was then washed three times in lysis buffer and boiled in electrophoresis sample buffer for 15 min. Samples were electrophoresed in $NaDodSO_4$/16.5% acrylamide gels ($NaDodSO_4$/PAGE) crosslinked with Acrylaide (FMC, Rockland, ME) using the buffer system of Laemmli (14), and autoradiography was performed as described previously (15). Desferrioxamine and hemin solutions were prepared as described previously (16).

**Cells.** Stable transformants were maintained in Eagle's minimum essential medium containing hypoxanthine/aminopterin/thymidine and supplemented with 10% heat-inactivated fetal bovine serum (GIBCO). Human K562 cells were grown as described previously (15).

**RNA Preparation and Evaluation.** Total RNA was prepared by solubilizing cells in guanidinium isothiocyanate and isolating RNA after ultracentrifugation through a 5.7 M CsCl cushion (17). Formaldehyde gels of 0.7% agarose were run, blot hybridization was performed (17), and probes from the 3′ coding region of the ferritin H chain were random-primed (18) to specific activities of $1–5 \times 10^8$ cpm per μg of DNA using [α-$^{32}$P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham).

Cytoplasmic RNA was prepared by lysis of cells in 0.1% Nonidet P-40/10 mM Tris, pH 8.5/1.5 mM $MgCl_2$/140 mM NaCl/10 mM vanadyl ribonucleoside complex. Cellular lysis and nuclear integrity were assessed, nuclei were pelleted, and the supernatant was mixed in a 5:1 volume ratio with 50 mM Tris/10 mM EDTA/10 mM NaCl/0.5% $NaDodSO_4$, pH, 8.0. A 30-min 37°C treatment with proteinase K (Boehringer Mannheim) at 50 μg/ml was followed by phenol extraction,

Abbreviations: H chain, heavy chain; L chain, light chain.

ethanol precipitation, and resuspension in sterile $H_2O$, and blot-hybridization analysis was performed.

**Manipulations of Ferritin mRNA Levels.** Sodium butyrate (Sigma) was added to the medium of plated cells 24 hr before harvesting by mechanical scraping. In experiments employing iron manipulation of butyrate-treated cells, desferrioxamine was added to medium containing butyrate at 16 hr and hemin at 4 hr before harvest. In the butyrate time course, butyrate was added to cells 72, 48, and 24 hr before harvest. Fresh medium containing butyrate was added each day.

## RESULTS

**Expression and Assembly of a Transfected Ferritin H-Chain Gene Product.** In a previous publication (12), we described the stable transfection of a genomic clone of the ferritin H-chain gene into mouse B6 fibroblasts. Analysis using S1 nuclease verified the expression of human H-chain message in these transformed cells. Further studies of these transfected cells have now been performed. To determine if human protein is made, ferritin was immunoprecipitated from biosynthetically labeled transformed cells (henceforth referred to as HfH transformants). Subsequent analysis on $NaDodSO_4$/PAGE revealed mouse H- and L-subunit chains and a third band of intermediate size, which represents the product of the transfected human H-chain gene. This band was not present in nontransfected mouse cells (Fig. 1*A*). Immunoprecipitates from lysates of human erythroleukemia (K562) cells (Fig. 1*A*, lane 1) performed with a polyclonal anti-human ferritin antibody showed one major band corresponding to the H chain and a poorly resolved lower band corresponding to the L chain. The human H-chain band in K562 cells comigrated with the new band present in the transfected cells. The human H-chain band appeared as the predominant ferritin band in the HfH transformants. Further proof that this represents the human H chain was provided by the use of a monoclonal antibody specific for human H chain (13). This antibody specifically precipitates ferritin from HfH transformants but not from control murine cells (Fig. 1*C*).

To determine if the human subunits were incorporated into ferritin polymers, the transfected cells were lysed and fractionated on a sizing column. After calibration of the column with horse spleen ferritin, immunoprecipitation of fractions of eluate was performed using the anti-human H-chain monoclonal antibody. Immunoprecipitates showed a peak of activity, which corresponds to the peak produced by native ferritin spheres derived from horse spleen. This demonstrates that all of the human ferritin detected by immunoprecipitation in these lysates was incorporated into high molecular weight molecules representing assembled ferritin (Fig. 1*B*). Furthermore, the human ferritin was able to co-assemble with mouse ferritin (Fig. 1*C*). To demonstrate co-assembly, the previously described monoclonal antibody specific for human ferritin was used to immunoprecipitate lysates from mouse fibroblasts, human K562 cells, and the HfH transformants. Immunoprecipitation of each cell type was also performed using a polyclonal antibody incapable of distinguishing murine from human ferritin. Mouse ferritin can be immunoprecipitated by the polyclonal antibody, but it was not precipitated by the anti-human monoclonal antibody (Fig. 1*C*, lanes 1 and 2). Human H and L subunits were present in immunoprecipitates of human K562 cells when either antibody was used (Fig. 1*C*, lanes 5 and 6). Immunoprecipitation of lysates from the HfH cells revealed a lower band corresponding to mouse ferritin when either the polyclonal or the monoclonal antibody was used. Since the monoclonal antibody does not recognize mouse ferritin, the presence of mouse subunits in the gel implies that the mouse subunits were precipitated because of their association in heteropolymers with human ferritin subunits. Thus the contact points

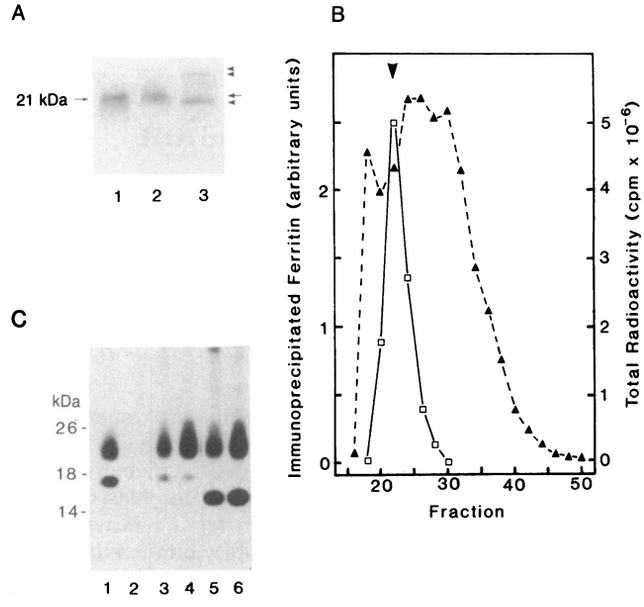


FIG. 1. Expression and assembly of transfected human ferritin H chain. (*A*) Expression of transfected human ferritin H chain. Human K562 cells (lane 1), HfH transformants (lane 2), or mouse fibroblasts (lane 3) were treated with hemin (50 $\mu$M) for 4 hr prior to metabolic labeling, lysis, immunoprecipitation with polyclonal anti-ferritin antibody, and $NaDodSO_4$/PAGE. Migration of mouse ferritin chains are denoted by arrowheads and that of the human ferritin H chain by the arrow on this autoradiograph of the 21-kDa region of the gel. (*B*) Assembly of transfected human ferritin H chain. HfH transformants were treated for 4 hr with hemin (50 $\mu$M) prior to metabolic labeling and lysis. The lysate (0.5 ml) was applied to an Ultrogel AcA 34 (LKB) column (1.0 × 26 cm) and was equilibrated in lysis buffer. Fractions of 0.55 ml were collected. Indicated fractions were assessed for their total radioactivity (▲) by liquid scintillation counting. Aliquots (0.4 ml) of alternate fractions were immunoprecipitated with a monoclonal anti-human ferritin antibody (13), and the immunoprecipitates were subjected to $NaDodSO_4$/PAGE, autoradiography, and quantitative densitometry. All detectable human ferritin (□) appeared in a peak between fractions 18 and 28. The peak migration position of horse spleen ferritin (Sigma) on the same column is indicated by the arrowhead. (*C*) Co-assembly of transfected human ferritin H chain and endogenous mouse ferritin. Mouse fibroblasts (lanes 1 and 2), HfH transformants (lanes 3 and 4), and human K562 cells (lanes 5 and 6) were treated for 4 hr with hemin (50 $\mu$M), metabolically labeled, lysed, and immunoprecipitated with either a polyclonal anti-ferritin IgG lacking species discrimination (lanes 1, 3, and 5) or with monoclonal anti-human ferritin H-chain antibody recognizing only human H chain (lanes 2, 4, and 6). Immunoprecipitates were subjected to electrophoresis on a 7.5% polyacrylamide gel using the phosphate/urea buffer system of Shapiro *et al.* (19). In this system, the human H chain and a mouse ferritin band comigrate in the 18-kDa to 26-kDa region, but another mouse ferritin band of lower apparent molecular mass is clearly resolved at approximately 17 kDa. Human ferritin L chain migrates at approximately 15 kDa (lanes 5 and 6). The lower molecular mass (17 kDa) mouse ferritin band is precipitated by the monoclonal anti-human ferritin antibody from lysates of HfH transformants (lane 4) but not from lysates of mouse fibroblasts (lane 2).

for assembly are similar enough between species to allow co-assembly between mouse and human subunits. There is a clear disparity between the relative intensities of the upper and lower bands in the parent mouse cells (Fig. 1*C*, lane 1) in comparison to the HfH cells (Fig. 1*C*, lanes 3 and 4). These data also suggest that the predominant ferritin species made in these transformants is human H chain (see below).

**Levels of Human Ferritin in HfH Transfectants.** The level of human ferritin H-chain expression in the HfH transformants is much higher than endogenous mRNA expression in host murine fibroblasts or in the nontransfected K562 cells. RNA

derived from mouse fibroblasts transfected by a gene unrelated to ferritin served as a control to demonstrate the amount of signal that was produced by hybridization to mouse ferritin mRNA (see Fig. 3*A*, lane 1). The signal obtained with RNA from these control cells remained low compared to human ferritin mRNA in the HfH transformants, even when the stringency of the hybridization and wash conditions was markedly reduced. This finding suggests that the mouse ferritin message levels are low compared to the human ferritin mRNA levels in the HfH cells and that low homology of the probe to mouse mRNA is not the cause of the decreased mouse signal (data not shown). When the mRNA levels of the HfH transformants are compared to ferritin mRNA levels from an equivalent amount of total RNA from human K562 cells, the signal from the transfected cells is generally 3–5 times higher. Southern blot analysis of total genomic DNA from the HfH cells confirms a high average copy number of 20–30 human ferritin genes (data not shown). Thus, the presence of high ferritin mRNA levels in the transfected cells can be explained, but it appears that the average mRNA production per introduced gene is lower than that seen with the endogenous gene of K562 cells.

**Regulation of Ferritin Biosynthesis.** A central feature of the biology of ferritin is the high degree of regulation of ferritin biosynthesis that takes place in response to changes in iron availability. We have, therefore, examined if the transfected human gene can produce a regulated gene product. Biosynthetic rates in the transfected cells were evaluated after drug treatments designed to perturb the iron status of the cell. Ferritin biosynthetic rates varied according to iron status; in the presence of hemin, the biosynthetic rate of ferritin was approximately 50 times higher than it was when a state of iron deprivation was induced (Fig. 2*A*). Total protein synthesis, measured by incorporation of [$^{35}$S]methionine into proteins, was unchanged by treatment with either hemin or desferrioxamine in these experiments, which attests to the selectivity of the effect upon ferritin synthesis. However, total mRNA levels for human H chains were not altered by changes in cellular iron status (Fig. 2*B*). Furthermore, when cytoplasmic RNA levels for human H chain were compared in cells treated with desferrioxamine or hemin, no differences were seen (Fig. 2*C*); thus, nuclear processing or nuclear transport as a source of regulation of biosynthesis is ruled out. Hemin has been shown to stimulate translation in reticulocyte lysates (20). In intact cells the effect of hemin or ferritin synthesis can be completely blocked by iron chelators, demonstrating that the hemin is functioning as an iron source (data not shown). In contrast to reticulocyte lysates, we do not see nonspecific increases in protein synthesis.

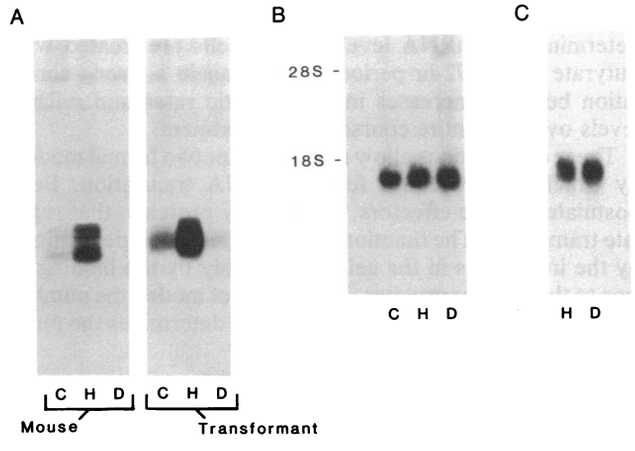


FIG. 2. Regulation of ferritin biosynthesis despite unaltered ferritin mRNA levels. (*A*) Regulation of ferritin biosynthesis. Mouse fibroblasts (*Left*) and HfH transformants (*Right*) were treated for 4 hr with 50 μM hemin (lanes H) or 16 hr with 50 μM desferrioxamine (lanes D) prior to metabolic labeling, lysis, immunoprecipitation with polyclonal anti-ferritin antibody, and NaDodSO$_4$/PAGE. The levels of newly synthesized ferritin are compared on this autoradiograph with those of untreated control cells (lanes C). (*B*) Blot-hybridization analysis of total RNA from HfH transformants. Total RNA was isolated and blot-hybridization analysis of 10 μg of RNA was performed. Cells were treated with hemin (lane H) or desferrioxamine (lane D) as described in *A* and are compared with untreated control cells (lane C). (*C*) Blot-hybridization analysis of cytoplasmic RNA from HfH transformants. Cells were treated with hemin (lane H) or desferrioxamine (lane D) as described in *A*. Cytoplasmic RNA was isolated and blot-hybridization analysis of 10 μg of this RNA was performed.

**Induction of Increases in mRNA Levels of the Ferritin H Chain Is Correlated with Increases in the Biosynthetic Rate.** When transfected cells were treated with various concentrations of sodium butyrate for 24 hr, increases in human ferritin H-chain mRNA levels were induced. Therefore, treatment of cells with sodium butyrate makes it possible to change intracellular mRNA levels in a controlled fashion and to evaluate the response of the cell to these altered mRNA levels. HfH transformants were treated with a range of concentrations of butyrate (0, 3, and 10 mM) and, for each butyrate concentration, treatments designed to alter iron availability were included. Human ferritin mRNA levels from cells treated with 50 μM desferrioxamine or 50 μM hemin were determined at each butyrate concentration. From 0 to 10 mM butyrate, levels of mRNA showed an increase of about 5-fold. Once again mRNA levels were clearly unaffected by iron status at each butyrate concentration (Fig. 3*A*). Rates of ferritin biosynthesis remained very responsive to cellular iron status and increased 10- to 20-fold as delivery of iron to the cells was increased (Fig. 3*B*). Although a range of iron response remained for each butyrate concentration, higher mRNA levels were correlated with higher protein biosynthetic rates at every point (Fig. 4). The correlation between mRNA levels and ferritin biosynthetic rates is also apparent in HfH cells receiving 3 mM butyrate for various times up to 72 hr. Levels of ferritin mRNA were elevated 3-fold after 24 hr and remained so for the duration of the treatment (Fig. 5). At all time points examined, the biosynthesis of ferritin closely reflected measured mRNA levels.

## DISCUSSION

We have demonstrated that stable transfection of the gene for human ferritin H chain into mouse fibroblasts results in the expression and regulation of an apparently normal and functional protein. All immunoprecipitable human ferritin chains are assembled into spheres of normal size, and at least some of these are heteropolymers that contain both human and mouse subunits. Increased availability of iron produced by treatment with hemin results in increased biosynthesis of ferritin, whereas intracellular iron depletion produced by treatment with desferrioxamine results in a marked decrease in ferritin biosynthesis. This regulation clearly occurs at a posttranscriptional level since cytoplasmic mRNA levels are not altered as a result of these treatments. Many previous reports have suggested that the regulation of ferritin levels is accomplished through regulation of translation (4–7). Although mRNA levels do not change in response to iron status, the amount of mRNA that is associated with ribosomes and is actively translated changes severalfold (7). We have recently demonstrated that the 5′ untranslated leader sequence of the ferritin H-chain mRNA is necessary for this regulation and is sufficient to confer the capacity for iron-dependent translational regulation when a hybrid mRNA is formed by ligation of this sequence to the 5′ end of an otherwise unregulated gene (unpublished results).

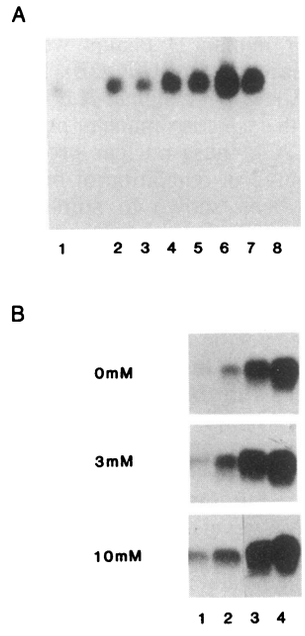


FIG. 3. Effect of sodium butyrate treatment of HfH transformants on ferritin mRNA levels and iron regulation of ferritin biosynthesis. (*A*) Effect of sodium butyrate treatment on ferritin mRNA levels. HfH transformants were treated for 24 hr without butyrate (lanes 2 and 3), with 3 mM butyrate (lanes 4 and 5), or with 10 mM butyrate (lanes 6 and 7). In addition, cells were treated for 16 hr with 50 $\mu$M desferrioxamine (lanes 2, 4, and 6) or for 4 hr with 50 $\mu$M hemin (lanes 3, 5, and 7). Total RNA was isolated and blot-hybridization analysis of 10 $\mu$g was performed. Lanes: 1, 20 $\mu$g of RNA from untreated mouse cells transfected with an unrelated gene; 8, 10 $\mu$g of total RNA from human K562 cells. (*B*) Effects of butyrate on ferritin biosynthesis and its iron regulation. Cells were treated for 24 hr with the indicated levels of butyrate as described in *A*. In addition, cells were treated for 16 hr with 50 $\mu$M desferrioxamine (lanes 1) or for 4 hr with hemin at 2 $\mu$M (lanes 2), at 10 $\mu$M (lanes 3), or at 50 $\mu$M (lanes 4). Biosynthetic labeling, immunoprecipitation with monoclonal anti-human ferritin H-chain antibody, $NaDodSO_4$/PAGE, and autoradiography were performed. The 20-kDa regions of these autoradiographs are shown. All data shown are derived from the same autoradiograph exposure.

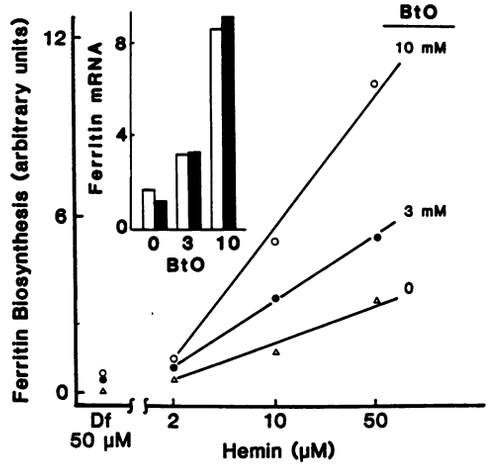


FIG. 4. Quantitative correlation between ferritin mRNA levels and ferritin biosynthesis. Autoradiographs of Fig. 3 *A* and *B* were quantitated by scanning densitometry. Relative rates of biosynthesis at 0 mM (△), 3 mM (●), and 10 mM (○) butyrate (BtO) are plotted as a function of hemin concentration, and the values obtained in desferrioxamine (Df)-treated cells are also shown. (*Inset*) Quantitation of ferritin mRNA at the indicated BtO treatment in HfH cells also treated with either 50 $\mu$M desferrioxamine (open bars) or 50 $\mu$M hemin (solid bars).

It is apparent that the level of biosynthesis of ferritin cannot be predicted from measurements of mRNA levels in the absence of information about the iron status of the cell. This fact is clearly demonstrated by the presence of 50- to 100-fold variations in protein synthesis rates in the absence of alterations in mRNA levels. To begin to understand the mechanisms underlying this regulation and to determine the points at which ferritin protein levels can be controlled, we have attempted to determine if the rate of transcription of the ferritin gene and the resulting mRNA levels can influence the level of ferritin protein synthesis. In a recent report, a number of different cell types were analyzed for levels of ferritin mRNA and ferritin protein. The results of this study suggested a direct correlation between mRNA levels and protein levels in different individual cell lines (21). However, individual cell lines may have other unmeasured metabolic features that account for this correlation between mRNA levels and biosynthesis. To evaluate the effect of altered mRNA levels on the translational regulatory apparatus, we have examined the regulatory response to induced increases in ferritin mRNA levels in a single cell line—the murine fibroblasts transformed by the human H-chain gene. Treatment of cells with sodium butyrate in millimolar concentrations has been correlated with the activation of many genes through a mechanism thought to involve inhibition of histone deacetylase and accumulation of hyperacetylated core particle histones (22). Southern analysis of genomic DNA from the transfected cell population indicates a high gene-copy number, which may account for elevated mRNA levels in these cells. When this cell population is treated with 10 mM butyrate, there is a 5-fold increase in ferritin mRNA levels, implying that transcriptional activity of the transfected genes increases in response to treatment with butyrate. Moreover, a dose-dependent increase in ferritin mRNA levels correlates directly with an increase in protein biosynthetic rate. Post-transcriptional regulation produced by perturbations in iron status is maintained, but the range of biosynthetic rates is determined by mRNA levels. When cells are treated with butyrate over a 72-hr period, there is again a strong correlation between increases in biosynthetic rates and mRNA levels over the entire course of the treatment.

These observations allow us to consider two formal models by which iron regulates ferritin mRNA translation. Both postulate specific effectors, presumably proteins, that regulate translation. The function of these effectors is determined by the iron status in the cell and possibly by the binding of iron to the effector proteins. In one type of model, the number of effector molecules stoichiometrically determines the num-

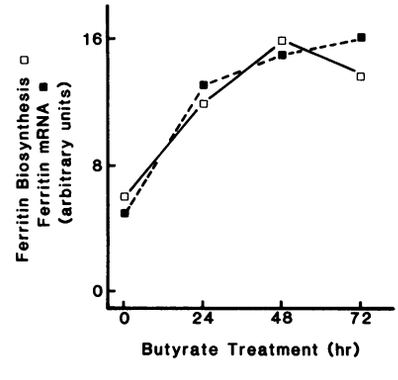


FIG. 5. Time course of the effect of butyrate on mRNA levels and ferritin biosynthesis. HfH transformants were treated with 3 mM butyrate for the indicated times. Relative ferritin mRNA levels (■—■) and ferritin biosynthetic rates (□—□) were quantitated from autoradiographs prepared as described in Fig. 3.

ber of actively translated mRNA molecules. The iron status determines the number of active effectors, and the absolute number of effectors is the sole determinant of the number of active mRNA molecules. In this model, the total level of ferritin mRNA beyond the number of effectors would be irrelevant to the level of ferritin biosynthesis. Such a model would predict no change in biosynthesis when mRNA levels increase and, therefore, is ruled out by our data. In the second type of model, sufficient effectors exist in the cell to distribute over all mRNA molecules and are, therefore, not limiting. In this type of model, iron determines the proportion of positive (or negative) effectors, which in turn determines the proportion of total mRNA that is translated. When we examine the data in Fig. 4, we see that for cells treated with either 0 or 10 mM butyrate (representing a 5-fold difference in mRNA levels) a 50% decrease in the biosynthetic rate is seen for each mRNA level when the hemin concentration is decreased from 50 to 10 $\mu$M. Thus our data demonstrate that a relatively constant ratio of ferritin biosynthetic rates is maintained between cells that express low (untreated) and high (butyrate-treated) mRNA levels, and this ratio remains constant over a wide range of changes in iron status. These studies suggest that, in addition to the high degree of translational regulation mediated by changes in iron status, the level of transcription of mRNA also determines the protein levels in the cell. Thus, the dynamic range of the cell for ferritin biosynthesis is determined by the level of ferritin mRNA (21). Within this range, the effects of cellular iron will be exerted at the level of translation. It appears that this potential source of regulation is employed in normal physiological settings. Studies of ferritin mRNA levels in differentiating HL60 cells have shown that absolute mRNA levels of H and L subunits rise and that H-to-L mRNA ratios increase substantially (23). These changes are reflected in differences in total ferritins and show that this increase in ferritin is achieved by way of regulation of mRNA levels.

The HfH transformants express markedly elevated amounts of ferritin H chain mRNA, and biosynthetic rates are high. What consequences does this have for the cell? In a previous study, we determined that the percentage of incoming iron that was sequestered by ferritin was determined by absolute intracellular ferritin levels (15). If increased biosynthetic rates result in increased intracellular ferritin levels, large amounts of incoming iron may be diverted away from normal metabolic pathways because of increased sequestration of iron by ferritin (24). Important features of intracellular iron metabolism may be revealed when the long term impact of elevated biosynthetic rates of ferritin is determined.

Transfection of the human H-chain gene into mouse fibroblasts has enabled us to construct a hybrid system in which the regulation of human gene products occurs in conjunction with the regulatory apparatus of the mouse cell. This system has allowed us to evaluate models that describe the molecular mechanisms of the regulation of ferritin biosynthesis. Iron does not affect ferritin message levels, and translational regulation in response to changes in iron status is determined by effectors that are sensitive to iron supply and determine the proportion of mRNA that is translated. Manipulations of mRNA levels produce proportional changes in biosynthetic rates in the absence of other perturbations, and these findings support the view that mRNA levels determine the range of biosynthetic rates that will be present when translational regulatory signals are integrated into the regulatory process. This observation may have important implications for other systems in which translational regulation is present. In the ferritin system, translational regulation is not a separate and distinct form of regulation but rather is intimately tied to transcriptional rates. A complex interdependence of transcriptional and translational regulation produces a responsive system of regulation, which can accommodate the metabolic requirements of cells in differentiation and proliferation.

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