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EVIDENCE FOR DISTINCT mRNAs FOR FERRITIN SUBUNITS

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SUMMARY: Poly A enriched RNA from iron loaded HeLa cells and rat liver were translated separately and together in wheat germ lysates to investigate the origins of the H and L subunits of ferritin. Most of the ferritin translated from the HeLa RNA was of the H type, while that from the liver RNA was mostly L type. Mixtures of these RNAs gave H/L ratios which correlated with the relative amounts of added HeLa and rat RNAs. These results indicate that the H and L subunits of ferritin are not derived by post-translational modification but from distinct mRNA species.

INTRODUCTION

Many tissues contain multiple forms of ferritin that differ structurally, immunologically and metabolically (1-4). These isoferritins appear to represent families of heteropolymers fashioned from different proportions of two polypeptides, H and L, in a 24 subunit shell (5). Differences in H and L production give rise to characteristic tissue ferritin phenotypes which vary with development, malignancy and iron overload (1,2). The two chains share extensive sequence homologies as evidenced by peptide mapping (5) but it is not clear whether they are derived from distinct mRNA species or by post-translational modification. This paper presents evidence that the H and L subunits arise from distinct mRNAs.

We have previously shown that both subunits can be identified in the translation products of wheat germ lysate programmed by poly A enriched RNA from rat liver (5). Consequently, we sought enrichments of putative mRNAs for both H and L to explore the production of these polypeptides in this system. Because H and L mRNA activities have not yet been separated,

EDTA, ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulphate; PBS, 137mM NaCl, 2.7mM KCl, 10mM phosphate, pH 7.4; PBT, 20mM phosphate, pH 7.4, 1% Triton X-100.

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we sought tissues that might provide a natural enrichment for such mRNAs. Iron loaded liver has a preponderance of L subunit (4) and was therefore chosen as a source of enriched L mRNA. A good source of H mRNA seemed more difficult since most normal tissues make smaller amounts of H than L. In addition, it is often difficult to extract active mRNA from those tissues such as heart, intestine and pancreas which have H rich phenotypes (1). The problem is compounded by the preferential production of L rich ferritins in these tissues (1,4) if they are loaded with iron in hopes of increasing ferritin mRNA levels. We therefore turned to HeLa cells for a natural enrichment of H mRNA since these cells produce H rich isoferritins, even in the face of iron loading (6). No H-rich rat cell line has been described yet.

MATERIALS AND METHODS

All reagents were analytical grade. L-[4,5-^{3}H (N)] Leucine, specific activity 58.5 Ci/mmol and Enhance, came from New England Nuclear, Boston, MA; Oligo d(T) cellulose Type 11 from Collaborative Research, Waltham, MA; Proteinase K from EM Biochemicals, Cincinnati, Ohio; ribonuclease A, Millipore Corp, Freehold, NJ; IgG Sorb from New England Enzyme Center, Boston,MA.
Tissues: Male Sprague-Dawley rats (125-150g) were fasted overnight and received 400 µg iron/100 g body weight 2 h before killing (4). HeLa cells were generously provided by Dr. E. Alpert, Mass. General Hospital, Boston. They were grown in Eagle's minimum essential medium supplemented with 10 µg iron/ml as ferric ammonium citrate for 72 h before harvesting(6).
RNA preparations: Rat livers were perfused in situ with 250mM sucrose, 1mM $MgCl_2$. Livers and HeLa cells (10-20g) were homogenized in 2 vols 0.1M Tris-HCl pH 7.4, 0.1M NaCl, 0.1mM EDTA, 5mM $MgCl_2$ in 0.25M sucrose containing 150 µg heparin/ml. Homogenates were centrifuged at 10,000 xg for 20 min. The resulting supernatant was filtered through glass wool and then centrifuged at 165,000 xg for 60 min. The microsomal pellet was suspended in 25 vols of 50mM Tris-HCl, pH 9.0, 150mM NaCl, 1mM EDTA, 0.5% SDS and 400 µg/ml Proteinase K. After 20 min incubation, RNA was extracted with phenol/chloroform/isoamyl alcohol and the poly A enriched fraction obtained by chromatography on oligo d(T) cellulose (7). After precipitation with 2 vol ethanol and 1/10 vol 2M NaCl, the RNA was dissolved in water at 0.25 mg/ml and stored at -80°.
Protein synthesis: Wheat germ lysates (8) were optimised for ferritin synthesis with respect to cation requirements and release of soluble polypeptides (5). Different proportions of HeLa and rat RNA preparations were mixed and translated at levels where protein synthesis was linear with respect to RNA input. Each reaction mixture of 100 µl contained a total of 2.5 µg of exogenous RNA and 20µCi ^{3}H leucine. After incubating at 30° for 90 min, the reaction mix was treated with ribonuclease (5), diluted with 200 µl PBS and clarified by centrifugation at 59,600 xg for 30 min.
H and L Synthesis: 0.75 µg of HeLa and rat liver ferritins (4,6) were added to the supernatant of the reaction mixture. This and the radiolabelled ferritin subunits were isolated immunologically by incubating

with a slight excess of antibodies to HeLa (1) and rat liver ferritins (4) for 1 h at 20° and 1 h at 4°. Immune complexes were recovered by incubating with an excess of IgG Sorb for 30 min at 20° followed by centrifugation at 8000xg for 2 min. The pellets were washed twice with PBT buffer then once with water. 7.5 µg each of rat liver and HeLa ferritins, was added as a marker for the H and L subunits and the mixture boiled for 10 min in SDS sample buffer for electrophoretic analysis (4). Under these conditions the H and L subunits from HeLa comigrate with those from rat liver so that only one H and one L subunit band were apparent in the stained gel. The gel was then treated with Enhance and subjected to fluorography (9). The relative incorporation into H and L was assessed by scanning the developed film in a Soft Laser Scanning Densitometer (Biomed, Chicago, IL) under conditions where intensity was linear with respect to incorporated radioactivity.

RESULTS AND DISCUSSION

The RNA from HeLa and rat liver both produced similar stimulation of ^{3}H leucine incorporation into proteins in the reaction mixture. However, the relative incorporation into ferritin compared with total protein translated from HeLa RNA was about twice that from the rat liver RNA, The relative incorporation into H and L subunits was also substantially different. In the HeLa RNA translation products, the relative incorporation was about 82% H and 18% L as compared to 30% H and 70% L from the rat liver RNA. These values are similar to the subunit composition of ferritins from iron loaded HeLa and rat liver cells (1). The relative intensities of the labelled H and L subunits synthesized from both RNA preparations did not change on prolonged incubation, indicating that the wheat germ lysate did not contain mechanisms for their interconversion. The relative incorporation into H and L was then determined from different proportions of added HeLa and rat mRNAs. In these experiments, the total protein synthesis from HeLa and rat RNAs was kept constant in order to control for possible modifications to H and L production by proteins other than ferritin which might be translated from either RNA preparation.

The fluorogram in Fig 1 shows the pattern of synthesis of H and L subunits programmed by different proportions of HeLa and rat RNAs. Comparison with the stained gel showed that the position of the labelled

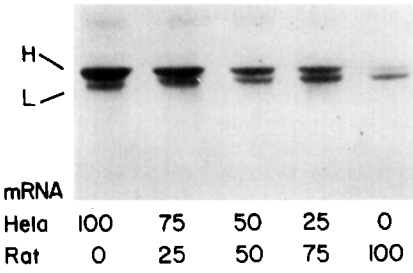

Fig. 1. Synthesis of H and L subunits programmed by poly A enriched RNA from HeLa cells and rat liver. HeLa and rat RNAs were mixed in the indicated proportions. After translation in wheat germ lysates, ferritin was immunoprecipitated from a volume corresponding to 1.19×10^6 dpm of acid-insoluble protein. The H and L subunits were separated electrophoretically and the fluorogram of the SDS gel was exposed for 2 days (see Methods).

subunits coincided with that of the marker subunits. Other analyses of dissociated immuneprecipitates by isoelectric focusing in urea gels also showed correspondence of radioactivity with authentic ferritin subunits. Both results indicate that neither the H or L subunit is made as a precursor (Watanabe, N. and Drysdale, J.W. in preparation).

Inspection of Fig 1 shows that the relative incorporation into H and L varied with the relative proportions of added HeLa and rat RNAs.

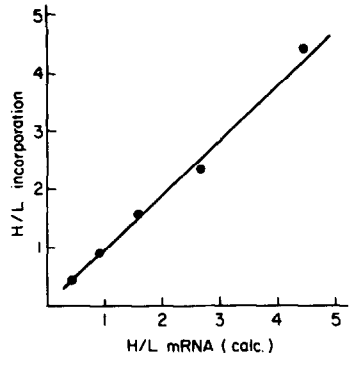

Fig. 2. Variation in incorporation of ^{3}H leucine into H and L subunits with different proportions of HeLa and rat liver RNAs. The relative synthesis of H and L was assessed from densitometric scans of the patterns in Fig 1 and are plotted against the calculated relative amounts of functional H and L mRNAs in the HeLa and liver RNA preparations.

Densitometric scans of the fluorogram showed that H/L ratios were essentially linear with the calculated proportions of H and L mRNA if it were assumed that the H/L ratio given by both RNA preparations reflected their relative content of H and L mRNAs (Fig 2).

These results indicate that the H and L subunits in human and rat ferritins are derived from distinct mRNA species rather than by post translational modification. Experiments are now underway to determine whether the 2 mRNA species are derived from different genes or by differential splicing from a common mRNA precursor as occurs for the membrane and secreted forms of the μ chain of IgM (10). Either mechanism would be of interest because of the differential effect of iron in promoting L synthesis in rat liver but H synthesis in HeLa cells.

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