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NATURAL ENRICHMENT OF FERRITIN mRNA IN mRNP PARTICLES

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SUMMARY: We have investigated the distribution of ferritin mRNA in polysomes and in mRNP particles from rat liver and HeLa cells by translation in wheat germ lysates. Our results indicate that the relative abundance of ferritin mRNA in both compartments is much higher than previously estimated. In mRNP particles from both tissues, ferritin appears to be one of the most abundant species.

INTRODUCTION

Many species of mRNA occur in inactive complexes with proteins in the cell sap [1]. In the case of the iron storage protein ferritin it has been suggested that these messenger ribonucleoprotein particles (mRNP) provide a reserve of ferritin mRNA which can be mobilized by iron to polysomes for the synthesis of apoferritin [2]. In an assessment by translational assay of the cytoplasmic distribution of ferritin mRNA in rat liver, it was reported that ferritin represented less than 0.2% of the protein synthesized from polysomal mRNA and 0.1-0.4% of the proteins programmed by mRNA from mRNP particles [2, 3]. In the course of our studies on the regulation of ferritin phenotypes, we have examined the apparent abundance of ferritin mRNA in polysomes and mRNP particles in rat liver and in HeLa cells. Our results indicate that the levels of ferritin mRNA in both compartments are about 20 times higher than the levels found by others. In both rat liver and HeLa mRNP particles, ferritin mRNA appears to be one of the most abundant mRNA species.

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 II from Collaborative Research, Waltham, MA; Proteinase K from EM Biochemicals, Cincinnati, Ohio;

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ribonuclease A, Millipore Corp., Freehold, NJ; IgG Sorb from New England Enzyme Center, Boston, MA.
Tissues: Livers were obtained from male Sprague-Dawley rats (125-150g) which had been fasted overnight. 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 [4].
RNA preparations: Polysomes and mRNP particles from normal rat liver and iron loaded HeLa cells were prepared as described by Zähringer et al. [3]. Poly(A) enriched RNA was prepared from these fractions as described elsewhere [4].
Protein Synthesis: Wheat germ lysates [5] were used to translate liver and HeLa mRNA in reaction volumes of 10-50 µl. After incubation for 90 min at 30° samples were treated with ribonuclease and assessed for incorporation of ^{3}H-leucine into acid-insoluble protein [4]. The distribution of radioactivity in total protein and in ferritin subunits was assessed by fluorography [6] following SDS gel electrophoresis. Incorporation into ferritin subunits was similarly assessed after immunoprecipitation with appropriate antibodies to rat heart and HeLa ferritins [4]. The following system was found to optimize resolution of ferritin subunits:-separating gels, 16.5cm high, contained 15% acrylamide and 0.6% N,N' methylene bis acrylamide (bis), 37.5% glycerol, 0.1% sodium dodecyl sulphate (SDS), 0.375M Tris-HCl, pH 8.8. The stacking gel, 2cm high, contained 3.94% acrylamide and 0.11% bis, 10% glycerol, 0.1% SDS and 0.125M Tris-HCl, pH 6.8. The running buffer was 0.05M Tris, 0.384M glycine and 0.1% SDS, pH 8.4. Samples were treated as before [4]. Gels were run for 3 h at 80v, then for 16 h at 150v when the tracking dye was 3cm from the bottom of the gels.

RESULTS

Figure 1 shows the translation products programmed by poly(A) enriched RNA from polysomes and mRNP particles from both rat liver and HeLa cells. A wide variety of peptides was synthesized from all four preparations. Comparison of patterns from homologous polysomal and mRNP fractions showed extensive similarities in the type and amount of proteins synthesized from both mRNA fractions. However, there were striking differences in the relative incorporation into ferritin synthesized from the mRNA from the polysomal and mRNP fractions. In this gel system, it was possible to obtain a good estimate of incorporation into the H and L subunits of ferritin [7] by direct densitometric evaluation of the fluorogram (Fig. 2). Of the translation products from rat liver RNA, ferritin represented about 5% of the total incorporation from polysomal mRNA and about 17% of the protein synthesized from the mRNP preparation. In the mRNP fraction, ferritin was the major translation product. A similar enrichment for ferritin mRNP was found from HeLa

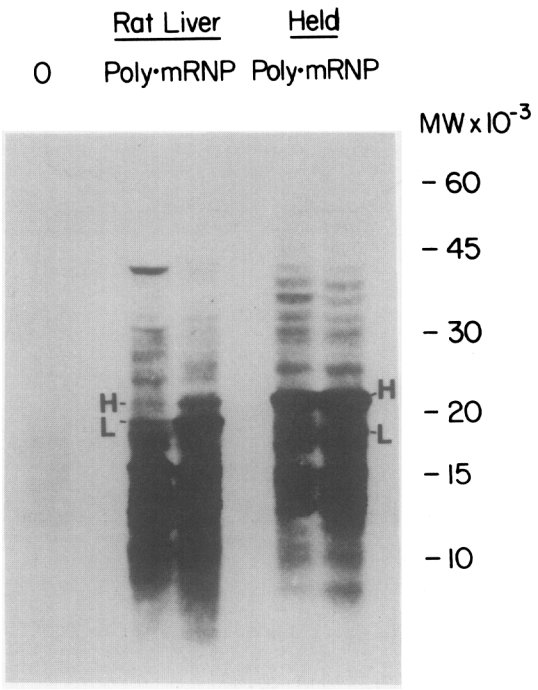

Fig. 1. Translation products from mRNA from polysomal (poly) and mRNP fractions of rat liver and HeLa cells. Poly A enriched RNA was translated in wheat germ lysates and the translation products displayed by fluorography after SDS gel electrophoresis.

cells. In HeLa polysomal RNA, ferritin synthesis accounted for 9% of the total protein synthesis while in the mRNA from the mRNP particles, ferritin represented about 25% of the total protein synthesis. These striking levels of ferritin synthesis and differences in mRNA distribution were confirmed by analyses of immuneprecipitates of ferritin subunits (Fig. 3). By this method, the relative incorporation into ferritin from the mRNA from mRNP particles of rat liver was approximately 6.5 times that given by polysomal RNA. In the HeLa preparations, the relative incorporation into ferritin from the mRNA of the mRNP particles was about 2 times that given by the polysomal mRNA. Essentially the same results were obtained from other preparations from rat liver and HeLa cells. In two other preparations from different liver pools, incorporation into ferritin averaged 3.7% for polysomal mRNA and 17.0% for

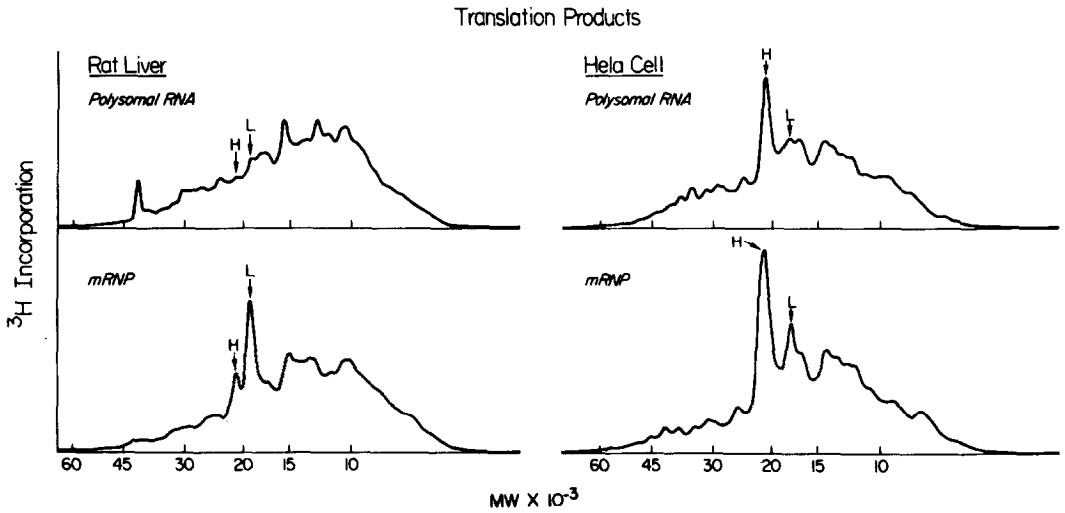

Fig. 2. Gel scans of translation products from rat liver and HeLa cells (See Fig. 1).

mRNA from mRNP particles. The corresponding figures for two other batches of HeLa cells were 11.2% and 20.6%.

In addition to the differences in the abundance of translatable mRNA for ferritin between polysomes and mRNP particles, there were also interesting differences between liver and HeLa cells in the apparent abundance of the H and L mRNAs. In both rat liver polysomes and mRNP particles, there was more translatable mRNA for L than for H. By contrast, the mRNA from polysomes and mRNP particles from HeLa cells coded almost entirely for the H type. In both rat liver and HeLa cells, there was no apparent difference in the relative proportions of H and L mRNAs between polysomes and homologous mRNP particles.

DISCUSSION

Our results indicate that ferritin mRNAs are among the more abundant species in polysomes from rat liver and HeLa cells. Of the mRNA in the mRNP particles, the relative proportion as ferritin is substantially higher, so much so, that ferritin mRNA appears to be the most abundant species of mRNA in rat liver mRNP particles. An enrichment for ferritin mRNA in these particles was first reported by Zähringer et al (2, 3).

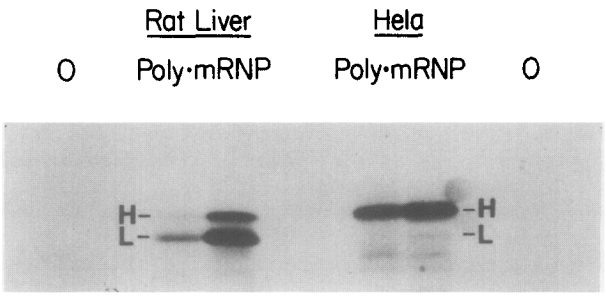

Fig. 3. Fluorogram of immuneprecipitated ferritin from translation products of Fig. 1.

However, they found that ferritin represented only 0.1-0.2% of the polysomal mRNA and 0.1-0.5% of the mRNA extracted from mRNP. Our results with a similar translation assay in wheat germ lysates indicate that ferritin mRNA is at least 20 times higher in both compartments. We think these discrepancies arise from technical problems in their method of relating radioactivity in immuneprecipitates from gel slices with that in the total translation products precipitated on a filter. Poor recoveries of ferritin subunits can also arise from immunological differences between the H and L subunits and also between subunits and shells (8). Non-specific losses also occur at various stages in electrophoresis and in recovery of radioactivity from the high percentage gels used to separate ferritin subunits. We have obtained similarly low values with this approach. However, we think that direct analyses by fluorography, despite its limitations, allows a more reliable assessment of relative incorporation into ferritin. The resolution in the gel is sufficient to identify both ferritin subunits for direct densitometric evaluation of the fluorogram. Similar results are also given at the higher resolution of 2D mapping and by gel analyses of immuneprecipitates when corrected for losses noted above. Translation in reticulocyte lysates gives similar results although, as expected, the general size distribution of other proteins is higher than in wheat germ (our unpublished results).

We prefer the wheat germ because of its less troublesome background from endogenous protein synthesis.

The question now arises as to whether translation of functional mRNA provides a good estimate of the relative abundance of ferritin mRNA in cells. In many cases where cDNA quantitation was also available, translation and hybridisation assays usually give similar results (10,11). Although cDNA probes for ferritin sequences are not yet available, we find that ferritin represents about 5% of the total protein synthesis in HeLa cells labeled in culture (our unpublished observations). Since this is similar to the value we find for ferritin mRNA by translation, we think that this value and the value of 25% in mRNP particles are likely to be good estimates. If so, the remarkable abundance of ferritin mRNA sequences in mRNP particles should greatly facilitate preparation of cloned DNA sequences and open the way for more definitive studies on the regulation of ferritin synthesis.

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