Immunopurification of the Suppressor tRNA Dependent Rabbit β-Globin Readthrough Protein†

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ABSTRACT: In mammalian cells, the rabbit β-globin readthrough protein is the only known example of a naturally occurring readthrough protein which does not involve a viral system. To provide an efficient means for its isolation, detection, and study, we elicited specific antibodies against this unique protein. The 22 amino acid peptide corresponding to the readthrough portion of this protein was synthesized, coupled to keyhole limpet hemocyanin, and injected into sheep. Specific antibodies to the peptide were produced as demonstrated by the enzyme-linked immunosorbent assay technique and by immunoblotting. The antibodies did not react with globin. The rabbit β-globin readthrough protein was separated from globin and other reticulocyte proteins by polyacrylamide gel electrophoresis and visualized by silver staining or by labeling with [^{35}S]methionine. Incorporation of [^{35}S]methionine into the readthrough protein was significantly enhanced upon addition of an opal suppressor tRNA to reticulocyte lysates. Immunoblotting revealed that the readthrough protein also occurs in lysates without added suppressor tRNA. The antibodies were purified on an affi-gel column which had been coupled with the peptide antigen. The readthrough protein was then purified from reticulocytes by immunoaffinity chromatography and by high-performance liquid chromatography. The results provide conclusive evidence that the β-globin readthrough protein is naturally occurring in rabbit reticulocytes.

There are several examples of natural suppression of termination codons in higher eucaryotes [see Hatfield (1985) for a review]. Synthesis of the murine and feline leukemia viral protease, reverse transcriptase, and nuclease depends on suppression of a UAG termination codon at the *gag–pol* readthrough site of viral mRNA by a host glutamine tRNA (Yoshinaka et al., 1985a,b). The glutamine tRNA involved in this readthrough has been sequenced from Moloney leukemia virus (MuLV)[1]-infected cells and has been found to be enhanced manyfold in these cells (Kuchino et al., 1987). Synthesis of the tobacco mosaic viral 183K readthrough protein, which is required for viral maturity, results from suppression of a UAG codon by an undermodified tyrosine tRNA (Beinz & Kubli, 1981; Beier et al., 1981, 1984). The rabbit β-globin readthrough protein (Geller & Rich, 1980) is, so far, the only example of a naturally occurring readthrough protein in mammalian cells which does not involve a viral system (Hatfield, 1985). This protein was detected as a labeled spot on an autoradiogram after incubating intact reticulocytes in the presence of [^{35}S]methionine and separating the resulting labeled proteins by polyacrylamide gel electrophoresis (Geller & Rich, 1980). Direct evidence that this spot is indeed a readthrough protein has not been provided.

Studies on the occurrence and cellular function of readthrough proteins in higher eucaryotes are extremely important, not only for providing a better understanding of the presence and function of these proteins but also for elucidating the cellular role of the suppressor tRNAs involved in their expression. Suppressor tRNAs have been used as models in gene therapy experiments to alleviate human diseases resulting from nonsense mutations (Temple et al., 1982). Furthermore, it has been proposed that altering the presence of suppressor tRNAs in cells infected with certain viruses may inhibit viral expression (Hatfield, 1985). In the present study, we have used immunochemical procedures to provide direct evidence that the β-globin readthrough protein is a natural component of rabbit reticulocytes. Additionally, production of this unique protein in reticulocyte lysates is dependent upon the presence of a suppressor tRNA. The antibodies which were elicited against this protein were used to detect and isolate both the naturally occurring and the suppressor tRNA-induced readthrough proteins.

Materials and Methods

Reagents. A peptide which is 22 amino acids in length (designated readthrough peptide) and which corresponds to the portion of the rabbit β-globin polypeptide that is assumed to result from readthrough (Efstratiadis et al., 1977) was synthesized and coupled to KLH protein by Peninsula Laboratories, Inc. Complete Freund's adjuvant was purchased from Sigma Chemical Co., nitrocellulose filter paper from Schleicher & Schuell, low molecular weight protein standards from Bethesda Research Laboratories (BRL), Aquasol scintillation fluid from New England Nuclear, polyacrylamide from International Biochemical, Inc., affi-gel-10 from Bio-Rad Laboratories, CNBr-activated Sepharose 4B from Pharmacia, and [^{35}S]methionine (specific activity 1020 Ci/mmol) from

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[1] Abbreviations: ELISA, enzyme-linked immunosorbent assay; MuLV, Moloney leukemia virus; KLH, keyhole limpet hemocyanin; cpm, counts per minute; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid.

Amersham. The components used in enzyme assays involving ELISA and immunoblotting were purchased from Kirkegaard and Perry, Inc. Rabbit reticulocyte lysates used in protein synthesis were purchased from BRL, and reticulocytes used for isolating the readthrough protein were prepared as previously described (Hatfield et al., 1979).

Antisera Preparation and Immunodetection. Antibodies were obtained from a sheep (Dorset–Rambouillet breed, 1-year-old male weighing 150 lb) which was initially immunized by intradermal and intramuscular injection at several sites with 2.1 mg of peptide–protein conjugate in 3.7 mL of 66% complete Freund's adjuvant. An identical booster injection was repeated 21 days later. Starting 21 days after the initial immunization, the sheep was bled at 21-day intervals. Antibodies were detected by the ELISA technique (Bustin et al., 1982) using phosphatase-labeled affinity-purified antibody to sheep (IgG [H+L] rabbit) and *p*-nitrophenyl phosphate as substrate. For immunoblotting, proteins and peptides were transferred from gels to 0.2- and 0.025-μm nitrocellulose paper, respectively, the paper was incubated with 1:100 antibody dilution, and the sheep antibodies were detected with phosphatase-labeled rabbit anti-sheep IgG using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates (Lin & Kasamatsu, 1983; Mendelson & Bustin, 1984).

Immunochromatography. For purification of specific IgG, 100 mg of the readthrough peptide was dissolved in 3.5 mL of 0.1 M sodium bicarbonate, pH 8.1, and attached to affi-gel-10 which had been prepared from a 3-mL slurry by techniques furnished by Bio-Rad Laboratories. Unreacted sites on the coupled affi-gel were blocked with 1.0 M ethanolamine, pH 8.0; 30 mL of antisera which had been adjusted to 0.2 M NaCl–0.2% Triton X-100 was mixed with the coupled affi-gel, the mixture was shaken overnight at 4 °C, the suspension was poured into a 0.5 × 10 cm column, the unbound components were removed from the column with a solution containing 1× PBS with 0.2% Tween 20 and 0.2 M NaCl, and the antibodies were eluted from the column in 0.2 M NaCl–0.1 M glycine, pH 2.9, dialyzed overnight against 4 L of 1× PBS, and stored at −20 °C. Purified antibodies from five such preparations were pooled and attached to 1 g of CNBr-activated Sepharose 4B particles by shaking a mixture of both components overnight at 4 °C in 0.1 M $NaHCO_3$, pH 8.0. The suspension was poured into a 0.5 × 10 cm column and the eluate passed through the column 2 additional times. Unreacted sites were blocked as above, the column was washed with 0.5 M NaCl–0.1 M $NaHCO_3$, pH 8.3, then with 0.5 M NaCl–0.1 M sodium acetate, pH 4.0, and finally in 1× PBS, and the column was stored at 4 °C until ready for use. The readthrough protein was isolated from 200 mL of packed rabbit reticulocytes (after removal of ghosts and adjusting to 0.2 M NaCl–0.2% Triton X-100) as follows: The lysate was mixed with the antibody-coupled CNBr-activated Sepharose 4B, the mixture shaken gently overnight at 4 °C and then poured into a column, and the column eluate passed back through the column as above. The column was washed in 100 mL of 1× PBS with 0.2% Tween 20 and then 100 mL of 1× PBS and eluted in 0.2 M NaCl–0.1 M glycine, pH 2.9. The eluate was lyophilized, redissolved in H_2O, adjusted to pH 2 with dilute trifluoroacetic acid (TFA), and applied to a 3.9 mm × 30 cm Waters (Milford, MA) μBondapak C_{18} column as described (Copeland et al., 1986). A Waters HPLC system using two pumps controlled with a Model 660 solvent programmer and monitored with a Model 450 variable-wavelength detector was used. A gradient of 0–60% acetonitrile containing 0.05% TFA was developed over 60 min of ambient temperature with a flow rate of 1 mL/min. One-milliliter fractions were collected and aliquots taken for immunoblotting. Fractions which contained material reactive with the antibody were then applied to the same column and eluted with a 25–50% acetonitrile gradient at 1 mL/min for 60 min. One-milliliter fractions were collected and aliquots taken for ELISA and for polyacrylamide gel electrophoresis. The developed gels were stained with silver or were used for immunoblotting.

Protein Synthesis and Electrophoresis. Protein synthesis was carried out with nuclease-treated rabbit reticulocyte lysates in the presence of [^{35}S]methionine with or without an opal suppressor tRNA as described (Diamond et al., 1981; Hatfield et al., 1982). Polyacrylamide gel electrophoresis of lysates was carried out as described (Diamond et al., 1981; Hatfield et al., 1982) with the exception that 15 μL of the reaction mixture containing 640 μg of protein, which corresponds to more than 500 μg of rabbit globin [see Lodish (1971) and references cited therein], was loaded into each well (wells were 1.1 cm in width) and the gel developed over a 25-cm distance by electrophoresis for 17 h at 160 V with two to three buffer changes. Developed gels were stained with silver (Morrissey, 1981) and photographed. Alternatively, after being fixed in the solvents used in silver staining, gels were rinsed in H_2O and sliced into 0.2-cm strips with a Bio-Rad gel slicer, and the gel strips were dissolved in Aquasol and counted in a scintillation counter. The percent suppression of the β-globin mRNA termination signal was determined from the number of [^{35}S]methionine counts present in the readthrough protein divided by the total number of [^{35}S]methionine counts present in globin and the readthrough protein. Protein was also transferred from developed gels to nitrocellulose paper for immunodetection (see above). The peptide and a set of protein standards were electrophoresed on 20% polyacrylamide gels [acrylamide: bis(acrylamide) ratio = 112.5:1).

Results

Preparation of the Antigen for Producing Antibodies Specific to the Readthrough Protein. The strategy for preparing antibodies which can be used to isolate the readthrough protein free of globin and other reticulocyte proteins was to use the carboxy-terminal portion of the readthrough protein which results from suppression of the UGA termination signal in rabbit β-globin mRNA as an antigen. The 3′ end of rabbit β-globin mRNA including the readthrough sequence and the two tandem UAA termination codons which provide a stop signal for the readthrough protein are shown in Figure 1. The corresponding protein which would result from translation of this sequence is also shown. The amino acid at the UGA termination signal of rabbit β-globin, which is indicated in the figure by a question mark, is not known. The 22 amino acid peptide which was chemically synthesized as an antigen for producing antibodies to the readthrough protein is designated in Figure 1 as synthetic peptide and in the text as a readthrough peptide.

Preparation and Purification of Antibodies to the Readthrough Peptide. To prepare antibodies against the readthrough peptide, the peptide was coupled to KLH, and the resulting conjugated protein was used to immunize a sheep. Antibodies were detected in the antisera 3 weeks after the initial immunization. As shown in Figure 2, the color developed in the ELISA assay was dependent both on antigen and on antibody concentration. Immune serum at dilutions of 1:100, 1:500, and 1:1000, as well as the affinity-purified Ig fraction, reacted with the readthrough peptide. Preimmune serum, which was used as a control, did not. Under conditions of the assay, the serum at 1:100 dilution could readily detect

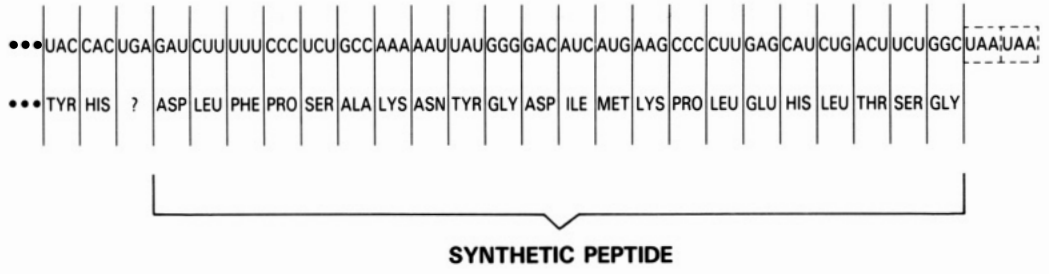

FIGURE 1: Sequence of the 3′ end of rabbit β-globin mRNA, the corresponding readthrough protein, and the synthesized peptide. The sequence of the 3′ end of rabbit globin mRNA is from the work of Efstratiadis et al. (1977). The mRNA contains a UGA termination codon, and the amino acid at the readthrough site of the corresponding polypeptide is unknown. The 22 amino acid peptide which was chemically synthesized and used to produce antibodies to the readthrough protein is shown under the nucleotide sequence.

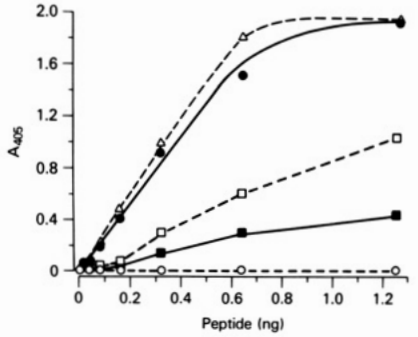

FIGURE 2: Immunodetection of the readthrough peptide by the ELISA technique. The wells of microtiter plates were coated in a total volume of 100 μL with increasing concentrations of peptide as shown on the abscissa. The bound antigen was reacted with antisera at dilutions of 1:100 (●—●), 1:500 (□---□), and 1:1000 (■—■) or with purified antibody at a dilution of 1:50 (△---△). Preimmune sheep serum was diluted 1:100 (○---○). The reaction was measured at A_{405nm} 20 min after addition of substrate, and the absorbance values were read with a Multiscan (Flow Labs).

200 pg of peptide. Longer color development further decreased the detection threshold.

Further evidence that the sheep produced antibodies specifically to the readthrough peptide was demonstrated by immunoblotting. The peptide was electrophoresed on a polyacrylamide gel along with a set of low molecular weight standards (Figure 3). Panel A depicts the Coomassie blue stain of the gel while panel B depicts the corresponding immunoblot.

Detection of the Readthrough Protein by Silver Staining and by ^{35}S Labeling. Before determination of whether the antibodies are suitable reagents for the specific detection of the readthrough protein, the occurrence of this protein in rabbit reticulocytes was examined by silver staining and by labeling with [^{35}S]methionine. Since the synthesis of the readthrough protein is presumably dependent on the suppression of a termination codon, the reticulocyte proteins were labeled with [^{35}S]methionine either in the presence or in the absence of exogenous suppressor tRNA. The lysates were fractionated on polyacrylamide gels and the proteins visualized by silver staining. The portion of the stained gel containing the globin and readthrough protein is shown in Figure 4A. The putative readthrough protein migrates just above globin in the gel and represents less than 1% of the globin. Addition of a suppressor tRNA did not produce any detectable change in the relative amount of readthrough protein as determined by silver staining. In contrast, examination of the autoradiogram of this gel (Figure 4B) clearly indicated that the synthesis of the readthrough protein is significantly increased by addition of suppressor tRNA. Quantitation of the level of suppression by scintillation counting of the areas containing the readthrough protein and globin (Figure 4C) indicated that addition

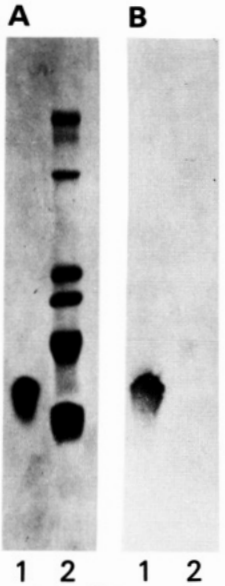

FIGURE 3: Western analysis of the readthrough peptide. Lane 1 contains 100 μg of peptide and lane 2 a set of low molecular weight protein standards (see Materials and Methods). (A) The developed gel stained with Coomassie blue and (B) an immunoblot following transfer of the peptide and the protein standards to nitrocellulose paper. Antisera were diluted 1:100.

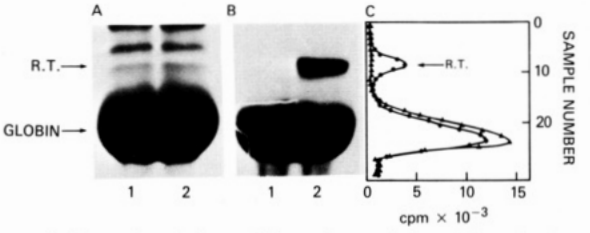

FIGURE 4: Detection of the readthrough protein in rabbit reticulocyte lysates by silver staining and by labeling with [^{35}S]methionine. Following protein synthesis, lysates were loaded onto a polyacrylamide gel, and the gel was developed as described under Materials and Methods. Only the region of the developed gel containing globin and the readthrough protein is shown. (A) Silver stain; (B) autoradiogram of (A); (C) quantitation of [^{35}S]methionine in globin and readthrough protein. Lanes 1 and 2 in (A) and (B) are lysate − suppressor tRNA and lysate + suppressor tRNA, respectively. In (C), cpm are designated on the abscissa and the number of gel strips on the ordinate, and lysate + suppressor tRNA is designated (●) and that without suppressor by (▲). 78 371 cpm were detected in globin and 401 cpm in the readthrough protein in lane 1 (−suppressor tRNA) and 63 746 cpm in globin and 9972 cpm in the readthrough protein in lane 2 (+suppressor tRNA). The positions of globin and readthrough protein (R.T.) are designated by arrows.

of suppressor tRNA elevated the level of the readthrough protein from 0.5% to 13.5% of the β-globin. Further evidence that the synthesis of the readthrough protein is stimulated by suppressor tRNA and that, in addition, the protein also occurs

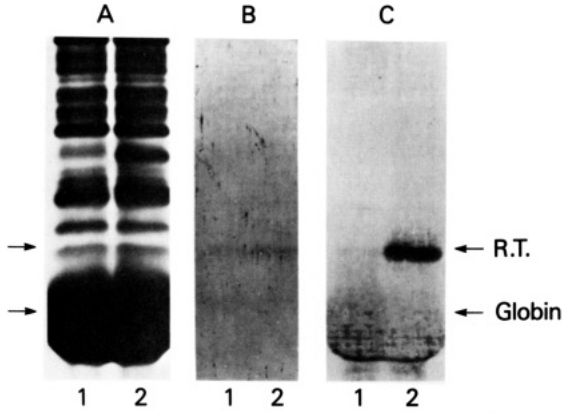

FIGURE 5: Immunodetection of the β-globin readthrough protein in rabbit reticulocytes. Following protein synthesis, lysates were loaded onto a polyacrylamide gel, and the gel was developed as described under Materials and Methods. In (A), lanes 1 (–suppressor tRNA) and 2 (+suppressor tRNA) were stained with silver; in (B), lanes 1 (–suppressor) and 2 (+suppressor) were immunoblotted with antisera which had been diluted 1:100; in (C), the immunoblot was then subjected to autoradiography. The amount of globin electrophoresed on polyacrylamide gels (see Materials and Methods) exceeded the capacity of the nitrocellulose filter; hence, most of the globin was lost during transfer from the gel to the filter which accounts for the low intensity of radioactivity observed in the globin band on the autoradiogram. The positions of globin and the readthrough protein (R.T.) are designated by arrows.

naturally in rabbit reticulocytes was obtained by immunological techniques (see below).

Immunodetection and Immunopurification of the Readthrough Protein. Evidence that the radioactive band stimulated by the suppressor tRNA is indeed the readthrough protein was determined by immunoblotting with the antipeptide antibody. Figure 5A presents the silver stain of the reticulocyte lysates incubated with and without suppressor tRNA and fractionated on polyacrylamide gels. A Western blot of the above gel (Figure 5B) reveals the presence of only one positive band which migrates in the same position of the readthrough protein. Autoradiography of the Western blot (Figure 5C) reveals that the immunodetected band was the only one whose synthesis was stimulated by the suppressor tRNA. Further examination of the immunoblot in Figure 5B indicates that the band was present also in the reticulocyte lysates incubated in the absence of exogenous suppressor tRNA. It seems, therefore, that the readthrough protein is a natural component of rabbit reticulocytes. To further verify the occurrence of the readthrough protein and to obtain further evidence that it reacts specifically with the antipeptide antibody, lysates incubated with suppressor tRNA and [^{35}S]-methionine were applied to affinity columns containing IgG purified from the antipeptide sera by adsorption onto peptide–Sepharose columns. The purified sample was compared to the unfractionated material by polyacrylamide gel electrophoresis. The silver stain presented in Figure 6A reveals that the affinity column indeed retained only a few of the proteins present in the lysate; however, the readthrough protein was still only a small fraction of the purified material. The autoradiograph of this gel (Figure 6B) reveals that whereas the unfractionated material contained several radioactive components the material recovered from the affinity column displayed only one radioactive band with a mobility identical with that of the readthrough protein. The two larger molecular weight bands in lane 2 of Figure 6A most probably are IgG components eluting from the column. The purification of the readthrough protein with respect to globin is very striking whereas in the unfractionated material the globin contained

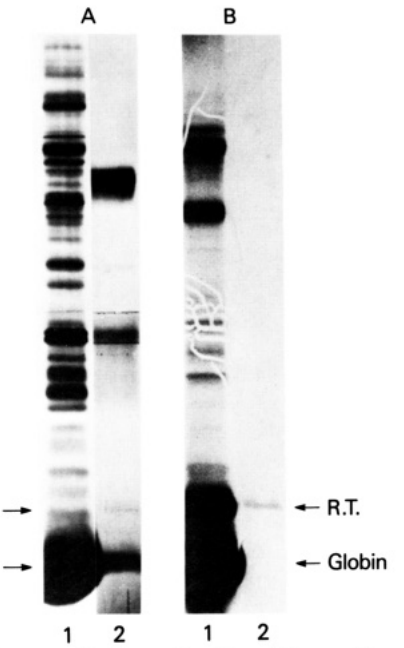

FIGURE 6: Immunoaffinity purification of the readthrough protein. A reticulocyte lysate was incubated with suppressor tRNA and [^{35}S]methionine and immunopurified on an affinity column containing antibodies for the readthrough peptide. In (A), lane 1 contains 10 μL of the reticulocyte lysate after protein synthesis, and lane 2 contains the material eluted from an affinity column after application of 100 μL of the same lysate to the column; both lanes were electrophoresed on polyacrylamide gels and then stained with silver. The photograph in lane 2 was exposed longer than that in lane 1 in order that the faint readthrough band could be observed. In (B), an autoradiogram of the silver-stained gel in (A) is shown. The positions of globin and the readthrough protein (R.T.) are designated by arrows.

significantly more radioactivity than the readthrough protein. The material recovered from the column did not contain any traces of radioactive globin. Examination of the stain in Figure 6A, lane 2, however, reveals a component migrating with the same mobility as intact globin. This component also reacts with the antipeptide antibodies (see Figure 7 below) and, therefore, contains the antigenic site for the readthrough portion of the readthrough protein. Although it was not labeled during incubation of the lysate with [^{35}S]methionine, this band probably reflects a degradation product of the readthrough protein which arises in the intact reticulocyte prior to the incubation period. To further purify the readthrough protein, the material obtained from the immunoaffinity column was chromatographed 2 successive times on HPLC. The fractions containing the readthrough protein were identified by ELISA. Figure 7 shows the purity of the material obtained after the second HPLC cycle and the purification steps selectively enriched for the readthrough protein. Silver staining revealed the presence of only two components, one with the mobility of the readthrough protein and the other with a mobility similar to globin (Figure 7A, lane 2). As mentioned earlier, the latter band most probably is a degradation product of the readthrough protein. Indeed, the immunoblot in Figure 7B reveals that both bands reacted with the antibody.

Discussion

A 22 amino acid peptide, which corresponds to the readthrough portion of the β-globin readthrough protein, was synthesized, coupled to KLH, and used to immunize sheep. Antibodies were produced to the readthrough protein as demonstrated by immunoblotting of rabbit reticulocyte lysates which had been electrophoresed on polyacrylamide gels and subsequently transferred to nitrocellulose filters and by im-

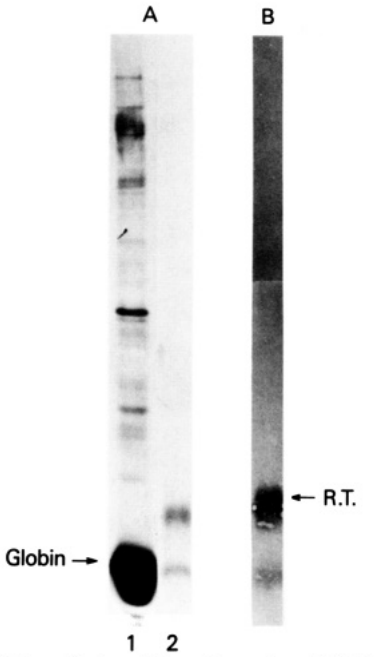

FIGURE 7: HPLC-purified readthrough protein. (A) Three microliters of a reticulocyte lysate (lane 1) and a sample of the readthrough protein purified by affinity chromatography on columns containing antibodies to the readthrough protein and by HPLC (lane 2) were electrophoresed on a polyacrylamide gel and stained with silver; (B) Western blot of (A). Affinity-purified antibodies were diluted 1:150. The lane containing the reticulocyte lysate did not show any response to the purified antibodies and, therefore, is not shown. The positions of globin and the readthrough protein (R.T.) are designated by arrows.

munopurification of the readthrough protein. It seems, therefore, that the antigenic site formed by the synthetic peptide is also present in the intact readthrough protein. The fact that the readthrough protein was observed in lysates which did not contain added suppressor tRNA demonstrates that this protein is a naturally occurring component of rabbit reticulocytes. In addition to immunoblotting, the readthrough protein was visualized on polyacrylamide gels by silver staining and by labeling with [^{35}S]methionine. The amount of readthrough protein which occurs naturally in reticulocytes was estimated to be approximately 0.5% as determined by the level of suppression of the β-globin termination signal in the absence of added suppressor tRNA. We estimate that by the combination of immunoaffinity chromatography and HPLC the readthrough protein has been purified over 1000-fold.

Our results clearly show that in vitro production of the readthrough protein is dependent on an opal suppressor tRNA. Furthermore, by using specific antibodies, we demonstrated unequivocally that the readthrough protein is indeed the elongation product of rabbit β-globin. The availability of antibodies specific to the readthrough protein will allow further studies on the cellular function and fate of this unique protein and on the opal suppressor aminoacyl-tRNA involved in its expression. As noted in the introduction, identification of a naturally occurring mammalian opal suppressor is of specific importance since nonsense suppressor tRNAs may provide avenues for gene therapy and a means of preventing viral expression. In addition, mammalian Middelburg and Sindbis viruses have a UGA codon in an open reading frame (Strauss et al., 1983, 1984) and a TGA codon has been detected in the mouse glutathione peroxidase gene, which occurs at the active site of the corresponding gene product (Chambers et al., 1986). Mouse glutathione peroxidase should not be considered as another example of a naturally occurring readthrough protein, since the UGA codon occurs in the open reading frame of the mRNA and the expression and function of the protein product depend on translation of this codon. It will be of interest to determine if the same opal suppressor tRNA that is involved in β-globin mRNA suppression in rabbit reticulocytes is also involved in suppression of some mammalian viral proteins and glutathione peroxidase.

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REFERENCES

Beier, H., Barciszewska, M., Krupp, G., Mitnacht, R., & Gross, H. (1981) *EMBO J. 3*, 351–356.

Beier, H., Barciszewska, M., & Sickinger, H.-D. (1984) *EMBO J. 3*, 1091–1096.

Bienz, M., & Kubli, E. (1981) *Nature (London) 294*, 188–190.

Bustin, M., Reish, J., Einck, L., & Klippel, J. H. (1982) *Science (Washington, D.C.) 215*, 1245–1247.

Chambers, I., Frampton, J., Goldfarb, P., Affara, N., McBain, W., & Harrison, P. R. (1986) *EMBO J. 5*, 1221–1227.

Copeland, T. D., Tsai, W.-P., Kim, Y. D., & Oroszlan, S. (1986) *J. Immunol. 137*, 2945–2951.

Diamond, A., Dudock, B., & Hatfield, D. (1981) *Cell (Cambridge, Mass.) 25*, 497–506.

Efstratiadis, A., Kafatos, F., & Maniatis, T. (1977) *Cell (Cambridge, Mass.) 10*, 571–585.

Geller, A., & Rich, A. (1980) *Nature (London) 283*, 41–46.

Hatfield, D. (1985) *Trends Biochem. Sci. (Pers. Ed.) 10*, 201–204.

Hatfield, D., Matthews, C. R., & Rice, M. (1979) *Biochim. Biophys. Acta 564*, 414–423.

Hatfield, D., Diamond, A., & Dudock, B. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6215–6219.

Kuchino, Y., Beier, H., Akita, N., & Nishimura, S. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 2668–2672.

Lin, W., & Kasamatsu, H. (1983) *Anal. Biochem. 128*, 302–306.

Lodish, H. L. (1971) *J. Biol. Chem. 246*, 7131–7138.

Mendelson, E., & Bustin, M. (1984) *Biochemistry 23*, 3459–3466.

Morrissey, J. H. (1981) *Anal. Biochem. 117*, 307–310.

Strauss, E., Rice, C., & Strauss, J. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 5271–5275.

Strauss, E., Rice, C., & Strauss, J. (1984) *Virology 133*, 92–110.

Temple, G., Doty, A., Roy, K., & Kan, Y. W. (1982) *Nature (London) 296*, 537–540.

Yoshinaka, Y., Katoh, I., Copeland, T. D., & Oroszlan, S. (1985a) *Proc. Natl. Acad. Sci. U.S.A. 82*, 1618–1622.

Yoshinaka, Y., Katoh, I., Copeland, T. D., & Oroszlan, S. (1985b) *J. Virol. 55*, 870–873.