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Opposite Responses of Rabbit and Human Globin mRNAs to Translational Inhibition by Cap Analogues†

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Received December 8, 1986; Revised Manuscript Received June 22, 1987

ABSTRACT: The translational efficiency of an mRNA may be determined at the step of translational initiation by the efficiency of its interaction with the cap binding protein complex. To further investigate the role of these interactions in translational control, we compare in vitro the relative sensitivities of rabbit and human α- and β-globin mRNAs to translational inhibition by cap analogues. We find that rabbit β-globin mRNA is more resistant to translational inhibition by cap analogues than rabbit α-globin mRNA, while in contrast, human β-globin mRNA is more sensitive to cap analogue inhibition than human α-globin mRNA. This opposite pattern of translational inhibition by cap analogues of the rabbit and human α- and β-globin mRNAs is unexpected as direct in vivo and in vitro comparisons of polysome profiles reveal parallel translational handling of the α- and β-globin mRNAs from these two species. This discordance between the relative translational sensitivities of these mRNAs to cap analogues and their relative ribosome loading activities suggests that cap-dependent events may not be rate limiting in steady-state globin translation.

Regulation of eukaryotic gene expression can be exerted in both the nucleus and the cytoplasm. While nuclear events, including transcription, processing, and transport, determine the pattern of mRNAs delivered to the cytoplasm, the final pattern of gene expression depends on the relative abilities of these mRNAs to direct protein synthesis. Studies in several systems now demonstrate that eukaryotic gene expression can be strongly influenced at the translational level [see, for example, Rosenthal et al. (1982), Babich et al. (1983), McGarry and Lindquist (1985), Reichel et al. (1985), and Warner et al. (1985); for a review, see Moldave (1985)]. One of the best-studied examples of translational regulation involves the balanced synthesis of α- and β-globin proteins in reticulocytes (Lodish, 1971, 1974). In both human and rabbit reticulocytes, the balanced synthesis of these proteins, which is critical to the normal development and function of red blood cells, occurs despite an excess of α-globin mRNA and appears to require more efficient translation of the less abundant β-globin mRNA [for a review, see Bunn et al. (1977)]. The differences in the translational activities of α- and β-globin mRNAs are reflected by differences in the distributions of these mRNAs in reticulocyte polysomes. In both rabbit and human reticulocytes, there is more efficient ribosome loading of β-globin mRNA compared to α-globin mRNA [rabbit (Hunt et al., 1968; Lodish, 1971); human (Clegg et al., 1971; Nathan et al., 1971; Boyer et al., 1974; Cividalli et al., 1974; Shakin & Liebhaber, 1986a)] as well as preferential sequestration of α-globin mRNA into translationally inactive complexes (Jacobs-Lorena & Baglioni, 1972; Shakin & Liebhaber, 1986a). Studies in rabbit reticulocytes suggest that the higher translational rate of β-globin mRNA results from a higher rate of β-globin translational initiation (Lodish, 1971).

The factors which control the efficiency of translational initiation in eukaryotic cells are still largely undefined. Re-

† This work was supported in part by Grant 1-RO1-AM-33975 from the National Institutes of Health. S.H.S. is a trainee in the Medical Scientist Training Program supported in part by Grant 5-732-GM-07170.

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0006-2960/87/0426-7188$01.50/0

cently, attention in this area has focused on interactions between mRNA and the cap binding protein complex. This complex appears to interact with the capped 5′ ends of eukaryotic mRNAs and enhance their ability to bind 43S ribosomal subunits [for reviews, see Shatkin (1985) and Pain (1986)]. Several studies suggest that cap binding proteins may serve to unwind secondary structures in the 5′ ends of mRNAs (Sonenberg et al., 1981; Lee et al., 1983; Edery et al., 1984; Ray et al., 1985). Both the accessibility of the cap structure (Godefroy-Colburn et al., 1985) and the degree of secondary structure in the 5′ ends of mRNAs (Gehrke et al., 1983; Edery et al., 1984) have been shown to influence the dependence of mRNAs on cap binding proteins for efficient translation. In particular, several studies suggest that the differences between the translational rates of rabbit α- and β-globin mRNA may reflect a difference in their cap dependence. First, the addition of cap structure analogues to translation reactions selectively reduces the synthesis of rabbit α-globin relative to rabbit β-globin (Suzuki, 1977), and, reciprocally, the addition of purified cap binding protein complex to translation reactions selectively enhances the synthesis of rabbit α-globin relative to rabbit β-globin (Sarkar et al., 1984). These findings suggest that the greater efficiency of rabbit β-globin translation may reflect a greater affinity of the rabbit β-globin mRNA for the cap binding protein complex or, alternatively, a lesser dependence of the β-globin mRNA on this complex for efficient translation.

Because of the similarities between the translational handling of the rabbit and human globin mRNAs in vivo, it seems likely that the mechanisms which regulate globin synthesis, as well as the structural signals in the α- and β-globin mRNAs involved in this regulation, are conserved between these two species. If the differential translation of α- and β-globin mRNAs reflects a difference in their relative dependence on cap binding proteins, one might expect that a progressive inhibition of the interaction of cap binding proteins with these mRNAs would result in a parallel shift in the relative rates of synthesis of rabbit and human α- and β-globin proteins. In this study, we test this hypothesis by directly comparing the selective translational handling of these mRNAs with their relative translational sensitivities to cap analogues.

Experimental Procedures

Isolation of RNA. Human globin mRNA was isolated from the blood of an adult with sickle cell anemia by phenol/chloroform extraction of acid-precipitated reticulocyte ribonucleoproteins as previously described (Liebhaber & Kan, 1982). Human poly(A+) mRNA was isolated by column chromatography of total reticulocyte RNA on oligo(dT)–cellulose (Maniatas et al., 1982). Rabbit 9S reticulocyte RNA was purchased from Bethesda Research Laboratories, Gaithersburg, MD.

3′ End Labeling and Analysis of Globin mRNAs. Human poly(A+) mRNA and rabbit 9S mRNA were 3′ end labeled with [5′-^{32}P]pCp (Amersham Corp., Arlington Heights, IL) and T4 RNA ligase (New England Biolabs, Beverly, MA) (Shaun et al., 1983) as previously described (Shakin & Liebhaber, 1986b). Labeled globin mRNA samples were electrophoresed on 3.5% acrylamide/8 M urea gels which were transferred onto Whatman 3M paper and autoradiographed without drying at −70 °C on XAR5 film (Eastman Kodak Co., Rochester, NY) with an intensifying screen (Lightning Plus; Du Pont Instruments, Wilmington, DE), all as previously described (Shakin & Liebhaber, 1986b).

Preparation and RNase H Analysis of mRNA/cDNA Hybrids. α- and β-globin mRNA/cDNA hybrids were prepared by hybridizing 3′ end-labeled rabbit and human globin poly(A+) mRNAs to either α-globin cDNA [human, pMC18 (Liebhaber & Begley, 1983); rabbit, pRCα1 (Heindell et al., 1978)] or β-globin cDNA [human, pSAR6 (Liebhaber et al., 1981); rabbit, pRCβ1 (Efstratiadis et al., 1977)]. Conditions for hybrid formation and subsequent analysis with RNase H (Bethesda Research Laboratories) (Stein & Hausen, 1969; Donis-Keller, 1979) were as previously described (Shakin & Liebhaber, 1986b).

In Vitro Translations. Translation reactions were carried out in micrococcal nuclease treated rabbit reticulocyte lysate (Pelham & Jackson, 1976) prepared from New Zealand White rabbits; 15-μL translation reactions were carried out at 30 °C for 30 min under previously described conditions (Liebhaber et al., 1984); 0.1 μg of either human poly(A+) mRNA or rabbit 9S mRNA was included in each translation reaction. Cap analogue (m^{7}GpppGm; Boehringer Mannheim, Indianapolis, IN) was resuspended in water to a concentration of 15 mM and stored at −70 °C. Cap analogue was diluted in water to concentrations of 0.075, 0.15, 0.30, 0.75, and 1.50 mM, and 1 μL of each cap analogue dilution stock was added to each of the indicated translation reactions to achieve the final concentrations indicated prior to the addition of lysate. For analysis of labeled proteins, 5 μL of each 15-μL translation reaction was electrophoresed on a 12% acrylamide Triton/urea gel (Rovera et al., 1978; Alter, 1979). Gels were fixed in 30% methanol/7% acetic acid twice for 1 h each time, impregnated with EN^{3}HANCE (New England Nuclear, Boston, MA), dried on Whatman 3M paper, and autoradiographed as described above.

Sucrose Gradient Fractionation and Analysis of in Vitro Translation Reactions. The sucrose gradient fractionation of in vitro translation reactions was performed as previously described (Liebhaber et al., 1984). Ten microliters of rabbit reticulocyte lysate without nuclease treatment was added to each translation reaction prior to sucrose gradient fractionation to enhance the UV absorption of monosome and polysome peaks. The labeled RNA in each isolated gradient fraction was phenol-extracted and ethanol-precipitated as described (Liebhaber et al., 1984) and analyzed by gel electrophoresis (see above).

Densitometric Analysis of Autoradiographs. Band intensities on autoradiographs were quantitated by using a soft laser scanning densitometer (Zeineh, Model SL-504-XL; Biomed Instruments, Fullerton, CA) with on-line computer integrating capacity. Autoradiographs were obtained at different exposure times so that the intensity of each scanned sample was within linear range. Where the peaks overlapped, each peak was traced onto filter paper and weighed for accurate quantitation.

Results

The polysome profiles obtained during translation of α- and β-globin mRNAs isolated from human and rabbit reticulocytes were compared in a nuclease-treated rabbit reticulocyte lysate in vitro translation system. Samples of rabbit and human reticulocyte mRNA were 3′ end labeled with ^{32}P prior to translation. After 30 min of translation, the distributions of the end-labeled α- and β-globin mRNAs (rabbit or human) were determined by fractionating each translation reaction on a sucrose gradient, resolving the α- and β-globin mRNAs in each gradient fraction by gel electrophoresis, and quantitating them by autoradiography. The identification and analysis of end-labeled globin mRNAs using this approach are shown in Figure 1, panels A and B. The two labeled bands in each RNA sample (rabbit or human) were identified as α-globin mRNA (bottom) and β-globin mRNA (top) by demonstrating

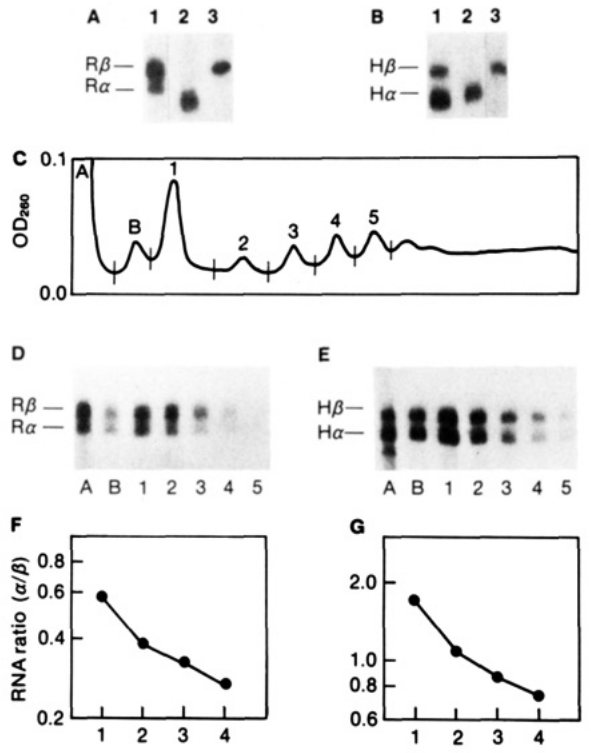

FIGURE 1: Relative ribosome loading of rabbit and human α- and β-globin mRNAs. (A and B) Identification of labeled rabbit and human α- and β-globin mRNAs by RNase H analysis. 3′-^{32}P end-labeled 9S rabbit reticulocyte mRNA (panel A) and 3′-^{32}P end-labeled poly(A+) human reticulocyte RNA (panel B) were mock-hybridized (lanes 1) or hybridized to β-globin cDNA (lanes 2) or α-globin cDNA (lanes 3); these RNAs were then digested with RNase H and analyzed by gel electrophoresis and autoradiography. The positions of rabbit α- and β-globin mRNAs (Rα and Rβ, respectively) and human α- and β-globin mRNAs (Hα and Hβ, respectively) are indicated. (C) Absorbance profile at OD_{260nm} of a sucrose gradient fractionated in vitro translation reaction. Rabbit reticulocyte lysate (without nuclease treatment) was added to the translation reaction prior to gradient fractionation as a UV absorbance marker of monosome and polysome peaks. Vertical lines indicate the extent of each isolated gradient fraction. Pre-80S fractions, A and B, and ribosome-associated fractions, 1–5, are indicated. Fractions labeled 1–5 contain mRNAs associated with one to five ribosomes per mRNA molecule, respectively. The top of the gradient is to the left. (D and E) Analysis of labeled α- and β-globin mRNAs in sucrose gradient fractions. Each gradient fraction was extracted and precipitated, and the labeled α- and β-globin mRNA content was analyzed by gel electrophoresis and autoradiography [panels D (rabbit RNA) and E (human RNA)]. The positions of rabbit and human α- and β-globin mRNAs are indicated. (F and G) Relative ribosome loading of rabbit and human α- and β-globin mRNAs. The ratio of α- to β-globin mRNAs in each of the ribosome-associated gradient fractions (1–4) shown in panels D (rabbit) and E (human) was quantitated by densitometric analysis and plotted on a semilogarithmic scale [panels F (rabbit) and G (human)].

that hybridization of the RNA to either α- or β-globin cDNA rendered the bottom or top band, respectively, fully sensitive to digestion with RNase H [Figure 1, panels A (rabbit) and B (human)]. The 3′ end labeling did not affect the overall translational activity of these mRNAs nor did it change the relative amounts of α- and β-globin proteins produced during in vitro translation (data not shown). The use of end-labeled RNA in this study was particularly useful, as it permitted the direct detection of the exogenous rabbit and human globin mRNAs with no background interference by endogenous rabbit globin mRNA fragments from the rabbit reticulocyte lysate. The relative labeling intensities of the α- and β-globin mRNAs detected on gel autoradiographs (Figure 1) were consistent with the relative concentrations of these mRNAs determined by independent hybridization analysis (data not shown). In the experiments described below, the ratio of α- to β-globin mRNAs in the rabbit reticulocyte mRNA sample was approximately 0.4, and the ratio of α to β-globin mRNAs in the human reticulocyte mRNA sample was approximately 1.0. The ratio of α- to β-globin mRNAs recovered during the isolation of poly(A+) RNA from total reticulocyte RNA can vary significantly from one mRNA preparation to another and does not reflect the actual ratio of these mRNAs present in reticulocytes.

A UV absorbance profile of an in vitro translation reaction fractionated on a sucrose gradient is shown in Figure 1, panel C. Fractions A and B which migrate above 80 S contain free globin mRNA, mRNA/40S complexes, 60S ribosomal subunits, and mRNPs with sedimentation densities less than 80 S. Fraction A also contains free hemoglobin which accounts for its high level of UV absorption. Fraction B is specifically enriched for 60S ribosomal subunits as confirmed by the detection of 28S rRNA on agarose gel electrophoresis (data not shown). Fractions 1–5 contain globin mRNAs associated with one to five ribosomes per mRNA molecule. In the experiments described in this report, mRNA was added to translation reactions at a subsaturating concentration (0.1 μg/15-μL translation reaction). The RNA extracted from each of the indicated gradient fractions of the in vitro translation reactions was analyzed by gel electrophoresis and autoradiography [Figure 1, panels D (rabbit RNA) and E (human RNA)]. The α- to β-globin mRNA ratios in the ribosome-associated fractions (1–4) were determined by densitometric analysis and plotted in Figure 1, panels F (rabbit RNA) and G (human RNA). A comparison of the ratios of α- to β-globin mRNAs in these lysate fractions demonstrates that β-globin mRNA from both species is enriched on heavier polysomes. The relative distributions of human α- and β-globin mRNAs during in vitro translation (Figure 1, panel G) were qualitatively the same when human poly(A+) reticulocyte RNA (isolated from the same individual) containing an α- to β-globin mRNA ratio of 2.0 was tested (data not shown), demonstrating that the relative distributions of these mRNAs in reticulocyte fractions is not dependent on their relative concentrations in the total translation reaction.

The relative sensitivities of α- and β-globin mRNA translation to inhibition by cap structure analogues were compared by translating rabbit and human globin mRNAs in the presence of different concentrations of cap analogue and determining the relative amounts of [^{35}S]methionine-labeled α- and β-globin proteins synthesized. The α- and β-globin proteins synthesized in each translation reaction were separated by electrophoresis on Triton/urea gels [Figure 2, panels A (translation of rabbit RNA) and B (translation of human RNA)] and quantitated by densitometric analysis of gel autoradiographs. In each gel, the upper band represents β-globin, and the lower band represents α-globin (Mezl et al., 1981). The β-globin doublet seen in the translation of rabbit globin mRNA (Rβ) represents the translation products of two co-dominant rabbit β-globin alleles (Rovera et al., 1978; Hardison et al., 1979). The minor band which migrates above human α-globin (Hα) represents an α-globin oxidation product (Liebhaber & Kan, 1983). Scans from the gel analysis of translation reactions containing no cap analogue and containing 0.1 mM cap analogue are shown for comparison [Figure 2, panels C (translation of rabbit RNA) and D (translation of human RNA)]. The relative ratios of α- to β-globin proteins produced in the absence of cap analogues (lanes 1) reflect in part the ratios of α to β-globin mRNA

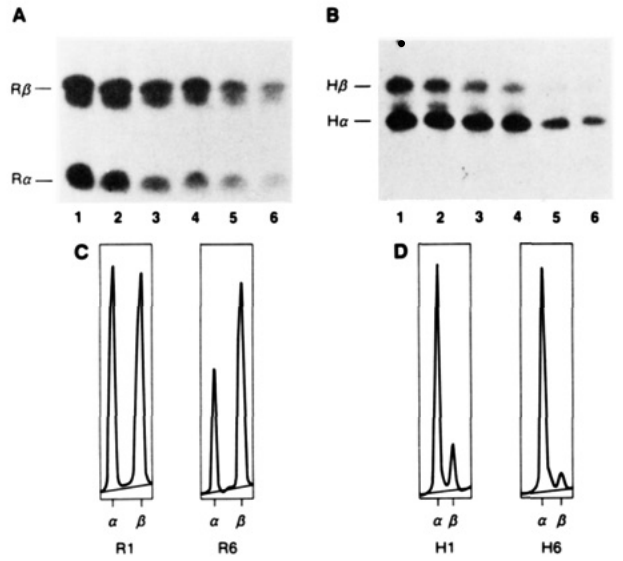

FIGURE 2: Effect of cap analogue on the translation of rabbit and human globin mRNAs. (A and B) 9S rabbit reticulocyte RNA (panel A) and poly(A+) human reticulocyte RNA (panel B) were translated in the rabbit reticulocyte lysate in vitro translation system, and the resulting [^{35}S]methionine-labeled globin proteins were analyzed by electrophoresis and autoradiography. Translations were carried out in the absence of cap analogue (lanes 1) or in the presence of cap analogue at final concentrations of 0.005, 0.01, 0.02, 0.05, and 0.1 mM (lanes 2–6, respectively). The positions of rabbit α- and β-globin proteins (Rα and Rβ) and of human α- and β-globin proteins (Hα and Hβ) are shown in panels A and B, respectively. (C and D) Representative scans obtained from densitometric analysis of the gel autoradiographs in panels A and B are shown in Panels C and D, respectively. The scans from translations of rabbit RNA and human RNA in the absence of cap analogue [R1 (panel C) and H1 (panel D)] and in the presence of 0.1 mM Cap analogue [R6 (panel C) and H6 (panel D)] are shown. The peaks representing labeled α- and β-globin proteins are indicated in each panel.

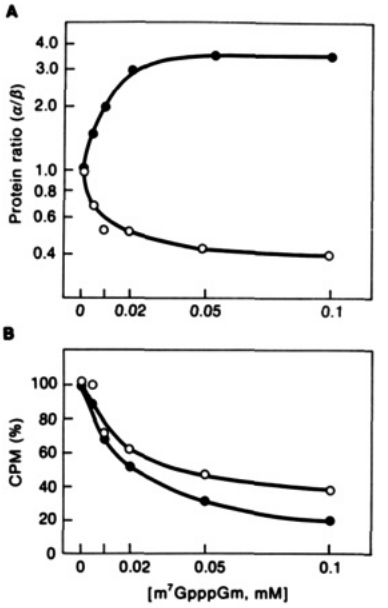

FIGURE 3: Relative sensitivities of rabbit and human α- and β-globin mRNAs to translational inhibition by cap analogue. (A) Effect of cap analogue on the ratio of α- to β-globin proteins produced during in vitro translation. The ratios of α- to β-globin proteins produced during in vitro translation in the presence of cap analogue (see Figure 2) were quantitated by densitometric analysis and normalized by dividing each ratio by the ratio of α- to β-globin proteins produced in the absence of cap analogue. These ratios are plotted as a function of cap analogue concentration for the translation of rabbit RNA (open circles) and human RNA (closed circles). (B) Inhibition of in vitro translation as a function of cap analogue concentration. The amount of TCA-insoluble [^{35}S]methionine-labeled protein produced (cpm) at each concentration of cap analogue was determined and expressed as a percentage of the total TCA-insoluble cpm produced in the absence of cap analogue (100%). These data are plotted for translations of rabbit RNA (open circles) and human RNA (closed circles).

added to the translation reactions (rabbit $\alpha/\beta = 0.4$; human $\alpha/\beta = 1.0$) as well as the number of methionine residues in each of the globin proteins [rabbit α- and β-globin and human β-globin each contain one methionine residue, while human α-globin contains two methionine residues (Dayhof, 1968)]. The ratio of α- to β-globin proteins produced in each of the translation reactions was divided by the ratio of α- to β-globin proteins produced in the translation reaction which contained no cap analogue (lanes 1); these normalized ratios are plotted in Figure 3, panel A. This plot reveals a relative enhancement of rabbit β-globin translation over that of rabbit α-globin translation in the presence of increasing concentrations of cap analogue. In contrast, the translation of human α-globin is relatively enhanced over that of human β-globin in the presence of increasing concentrations of cap analogue. It should be emphasized that this plot shows the relative ratios of α- and β-globin proteins produced and does not represent the absolute levels of the four globin proteins (human and rabbit α- and β-globin) synthesized; the absolute level of synthesis of each of these proteins was reduced as the concentration of cap analogue was increased (see Figure 2, panels A and B). The degree of overall translational inhibition at each cap analogue concentration was determined from scintillation counts of the trichloroacetic acid (TCA)-precipitated [^{35}S]methionine-labeled proteins produced in each translation reaction. These data, normalized to the amount of translation in the absence of cap analogue (100%), reveal that the translations of rabbit and human RNAs were inhibited to comparable degrees at concentrations of cap analogue up to 0.02 mM (Figure 3, panel B), the concentrations at which an effect of the cap analogue on the relative translational levels of the α- and β-globin proteins was observed. At concentrations of cap analogue higher than 0.05 mM, there was no further change in the relative amounts of α- and β-globin proteins produced (Figure 3, panel A), even though a further decrease in the absolute level of synthesis of these proteins was observed (panel B).

Discussion

In normal reticulocytes, the synthesis of α- and β-globin proteins is balanced despite an excess of α-globin mRNA. This balancing of globin gene expression is critical, since the accumulation of uncomplexed α- or β-globin chains is toxic to red cells and results in a hemolytic anemia [for a review, see Weatherall and Clegg (1981)]. Comparisons of globin gene expression in rabbit and human reticulocytes have revealed striking similarities. In both species, the relative ratios of α- and β-globin mRNAs produced are similar, as is the balancing of α- and β-globin synthesis [for a review, see Bunn et al. (1977)]. The polysome profiles of rabbit and human globin mRNAs in vivo are also similar, revealing both preferential sequestration of α-globin mRNA into translationally inactive complexes (Jacobs-Lorena & Baglioni, 1972; Shakin & Liebhaber, 1986a) and more efficient ribosome loading of β-globin mRNA [rabbit (Hunt et al., 1968; Lodish, 1971); human (Clegg et al., 1971; Nathan et al., 1971; Boyer et al., 1974; Cividalli et al., 1974; Shakin & Liebhaber, 1986a)]. These functional similarities suggest that the regulation of globin gene expression in rabbit and human reticulocytes at the translational level may involve similar mechanisms. Previous studies have suggested that the relative translational levels of these mRNAs may be governed by their relative rates of translational initiation (Lodish, 1971, 1974) and that their

translational initiation rates may in turn be a function of their relative dependence on the cap binding protein complex (Suzuki, 1977; Sarkar et al., 1984). In the present report, we tested this hypothesis by comparing the relative cap sensitivities of α- and β-globin mRNAs derived from rabbit and human reticulocytes in parallel in a rabbit reticulocyte lysate in vitro translation system. We first demonstrated that this translational system was appropriate for these studies by demonstrating that when exogenous rabbit and human α- and β-globin mRNAs were translated in this system they established the same relative polysome loading profiles as they do in vivo. This result suggests that the mechanisms which govern the translational handling of α- and β-globin mRNAs in vivo in rabbit and human reticulocytes are preserved cross-species and were preserved in the preparation of this in vitro translation system. The translational studies in this report were carried out at concentrations of mRNA which were subsaturating with respect to translation. These conditions were selected so that the data would specifically reflect the dependence of the globin mRNAs on the cap binding protein complex and would minimize the effects of competition by these mRNAs for other factors in the lysate. The results of the cap analogue translational inhibition study using rabbit reticulocyte mRNA, which showed that rabbit α-globin mRNA was more sensitive to inhibition by cap analogues than rabbit β-globin mRNA, are in agreement with the findings of previous studies (Suzuki, 1977; Sarkar et al., 1984). These results suggest that the higher rate of rabbit β-globin mRNA translation in reticulocytes may reflect a greater ability of this mRNA, compared to that of rabbit α-globin mRNA, to compete for the cap binding protein complex or, alternatively, that the translation of rabbit β-globin mRNA is less dependent on this complex for efficient translation. In contrast, we find that the human α- and β-globin mRNAs have the opposite relative sensitivities to cap analogue inhibition, with the translation of human β-globin mRNA more strongly inhibited by cap analogues than that of human α-globin mRNA. These findings suggest that the rabbit and human α- and β-globin mRNAs differ from one another in their relative dependence on the cap binding protein complex for efficient translation under conditions in which this complex is limited in availability. It is not clear, however, what role such differences play under normal translational conditions. Since the rabbit and human globin mRNAs are handled similarly to one another during normal translation (both in vivo and in this in vitro system), it seems unlikely that the opposite responses of these mRNAs to translational inhibition by cap analogues reflect processes normally rate limiting in their translational control.

There are several possible reasons why the relative sensitivities of these mRNAs to translational inhibition by cap analogues might not reflect their normal relative translational activities. First, in the absence of cap analogue, the cap binding protein complex may not be limited in quantity; therefore, differences in the dependence of the α- and β-globin mRNAs on this complex might not be apparent under normal translation conditions. Second, while cap-dependent reactions may be critical to the efficient translation of these mRNAs, some other more distal step in their translation may be rate limiting and may determine their individual ribosome loading and translational rates. For example, the rate at which ribosomes bind to mRNAs during active translation may be limited not by the efficiency with which 80S ribosomes are assembled (which may be cap dependent) but rather by the rate at which each newly assembled 80S ribosome is released from the initiation site, making room for the next ribosome binding event. Alternatively, mRNAs may be dependent on cap binding proteins for initial ribosome binding, while subsequent ribosome binding events during steady-state translation may be controlled by cap-independent mechanisms (Asselbergs et al., 1978). For example, the initial interactions of an mRNA with cap binding proteins may alter its secondary and higher order structure and, in doing so, may relieve its dependence on cap binding proteins for subsequent rounds of translational initiation. Consideration of such mechanistic differences between primary and subsequent ribosome binding events may help to clarify the roles of cap-dependent and cap-independent processes in translational regulation.

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Determination of the Acyl Chain Specificity of the Bovine Liver Phosphatidylcholine Transfer Protein. Application of Pyrene-Labeled Phosphatidylcholine Species†

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Received February 19, 1987; Revised Manuscript Received June 22, 1987

ABSTRACT: The phosphatidylcholine transfer protein from bovine liver has specific binding sites for the *sn*-1 and *sn*-2 acyl chains of the phosphatidylcholine molecule [Berkhout, T. A., Visser, A. J. W. G., & Wirtz, K. W. A. (1984) *Biochemistry 23*, 1505–1513]. In the present study, we have investigated the properties of these binding sites by determining both binding and transfer of several sets of pyrenylphosphatidylcholine species. These sets consisted of positional isomers in which the length of the pyrene-labeled acyl chain (i.e., 5–13 methylene units) or of the unlabeled saturated acyl chain (i.e., 9–19 methylene units) was varied in either the *sn*-1 or the *sn*-2 position. Binding studies showed that there was a considerable discrimination between positional isomers with the higher affinity observed for those lipids that carry the pyrenyl chain in the *sn*-2 position. In addition, the affinity is markedly dependent on the length of the acyl chains; pyrenyl acyl chains of 9 and 11 methylene units and the palmitoyl chain provided the most efficient binding. The affinity of the transfer protein for the strongest bound pyrene lipid was approximately 2.5 times higher than for an average egg phosphatidylcholine molecule. In general, the transfer studies were in agreement with the binding data. However, with some short-chain derivatives, transfer rates were faster than expected on the basis of the binding data. This emphasizes the importance of kinetic factors (i.e., activation energy) in the transfer process. The rates of spontaneous transfer decreased monotonically with increasing chain length and were very similar for all positional isomer pairs studied. This strongly suggests that the discrimination between isomers in the protein-mediated transfer reflects the structure of the lipid binding site rather than differences in the physical properties of the isomers. In conclusion, the present data strongly support the presence of separate binding sites in the transfer protein for the *sn*-1 and *sn*-2 chains of phosphatidylcholine and indicate that these binding sites have considerably different acyl chain specificity.

Phospholipids and cholesterol are known to transfer between membranes and lipoproteins (Bell, 1978; Norum et al., 1983). Transfer may take place spontaneously by diffusion of monomers through the aqueous phase (Martin & MacDonald, 1976; Duckwitz-Peterlein et al., 1977; Doody et al., 1980; Roseman & Thompson, 1980; McLean & Phillips, 1981; Nichols & Pagano, 1981) or is mediated by specific proteins found in cells (Wirtz, 1982; Kader et al., 1983; Zilversmit, 1984) and serum (Ihm et al., 1980; Morton & Zilversmit, 1982; Tall et al., 1983; Abbey et al., 1985). The mode of action of some of these lipid transfer proteins has been studied in considerable detail [for reviews, see Wirtz (1982), Helm-

† This research was supported by grants from The Finnish Academy, the European Molecular Biology Organization (EMBO), and the Federation of Biological Sciences (FEBS) to P.J.S. Part of this research was carried out under the auspices of The Netherlands Foundation for Chemical Research (SON) and with financial aid from The Netherlands Organization for the Advancement of Pure Research (ZWO).
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0006-2960/87/0426-7193$01.50/0