Short Half-Life Precursor of Globin Messenger RNA from Chicken Erythroblasts†

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ABSTRACT: The principal aim of this work was to investigate newly synthesized chicken globin RNA sequences. Erythroblasts, obtained from the bone marrow of anemic chickens, were pulse labeled with [^{3}H]uridine, and total cellular RNA isolated. The main findings reported here are that ^{3}H-pulse-labeled erythroblast RNA contains a 14S precursor to cytoplasmic 10S globin mRNA, and that the precursor has a short half-life of about 3 min at 39 °C. Properties of this RNA species satisfy the criteria for a functional higher molecular weight precursor to polysomal globin mRNA. (1) It is higher in molecular weight than polysomal globin mRNA; (2) it is newly synthesized; (3) the precursor is processed to 10S globin mRNA. In addition the precursor binds to oligo(dT)-cellulose, and so is polyadenylated. Newly synthesized globin RNA sequences were detected by cDNA-excess hybridizations to different size-class fractions of erythroblast RNA, separated by sucrose gradient centrifugation. Globin RNA-excess hybridization demonstrated that steady-state globin mRNA within this gradient was centered around 10 S. Hence the 14S globin RNA species is not a product of 10S globin RNA aggregation. RNA-excess hybridizations were also used to probe for globin RNA sequences in the 20–50S size range. In contrast to other reports (Imaizumi, T., et al. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70,* 1122; Spohr, G., et al. (1976) *Cell 8,* 505; Williamson, P. L., & Tobin, A. J. (1977) *Biochim. Biophys. Acta 475,* 366) no 20S–50S globin RNA sequences were detected.

It has been proposed that mRNAs in eukaryote cells are derived by posttranscriptional modification of larger RNA precursor molecules (Attardi et al., 1966; Scherrer et al., 1966; Darnell et al., 1973). Although HnRNA[1] has some properties expected for precursors to eukaryote mRNAs, much of the evidence for a precursor–product relationship is circumstantial. Poly(A) sequences are located at the 3′ end of most eukaryote polysomal mRNAs (Edmonds et al., 1971; Lee et al., 1971) and are found on a proportion of HnRNA molecules (Edmonds et al., 1971; Derman & Darnell, 1974). In addition, the turnover rate of HnRNA is about that expected for mRNA precursor RNA (Penman et al., 1968; Scherrer et al., 1970).

Perhaps the most definitive approach to investigating whether HnRNA contains higher molecular weight precursors to polysomal mRNAs is to study mRNA sequences of defined coding specificity that are produced in abundance in a particular cell type. DNA sequences complementary to a defined mRNA can then be used to probe RNA fractions of known molecular weight.

While this approach has been widely used, the results of several different groups have led to different conclusions. In particular there appears to be some disagreement on the size of higher molecular weight globin RNA sequences within avian erythroid cells. On one hand it has been proposed that avian globin sequences are contained within RNA molecules of several different size classes, ranging up to about 3×10^6 in molecular weight (Imaizumi et al., 1973; Spohr et al., 1976). In contrast, Macnaughton et al. (1974) only detected globin sequences in duck erythroid HnRNA about three times the size of polysomal globin mRNA.

The work described here has adopted a hybridization approach which specifically detects newly synthesized globin RNA sequences within avian erythroid cells. It demonstrates that chicken polysomal globin mRNA is derived from a functional precursor, which is about twice the size of steady-state globin mRNA.

This precursor is similar in size to those detected in mouse erythroid cells, but it has a much shorter half-life (3 to 4 min) compared with its counterpart in both mouse fetal liver cells (45 min, Ross, 1976), and Friend erythroleukemic cells induced by dimethyl sulfoxide (8 to 10 min, Curtis & Weissman, 1976).

The 14S chicken precursor binds to oligo(dT)-cellulose and is therefore likely to contain a 3′-poly(A) tract.

Experimental Section

Materials

Ribonuclease A (pancreatic), ribonuclease T_1, and unlabeled deoxynucleotide triphosphates were purchased from Sigma Chemical Co. All media were from Commonwealth Serum Laboratories (Melbourne, Australia). Oligo(dT)$_{12-18}$ was from P-L Biochemicals. AMV reverse transcriptase was provided by Dr. M. A. Chirigos and Dr. J. W. Beard.

Isotopes were obtained from the following sources: [α-^{32}P]dCTP (20–40 Ci/mmol) was a gift from Dr. R. H. Symons and 5,6-[^{3}H]uridine (30.7 Ci/mmol) was obtained from ICN, Irvine, Calif.

Methods

Cells and Cell Incubations. Erythroblasts were prepared from the bone marrow of anemic chickens as described by Appels et al. (1972). For the in vitro incorporation of [^{3}H]-uridine, erythroblasts were washed in NKM (0.15 M NaCl, 0.005 M KCl, 0.002 M $MgCl_2$), and incubated in Eagles basal medium containing 10% chicken serum (freshly prepared) and buffered with bicarbonate/CO_2 to pH 7.0. [^{3}H]Uridine solutions were dried down within the incubation vessel prior to adding incubation medium. Cells were at a concentration of

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[1] Abbreviations used: HnRNA, heterogeneous nuclear ribonucleic acid; NKM, 0.15 M NaCl, 0.005 M KCl, 0.002 M $MgCl_2$; SET, 2% sodium dodecyl sulfate, 0.01 M EDTA, 0.02 M Tris-Cl.

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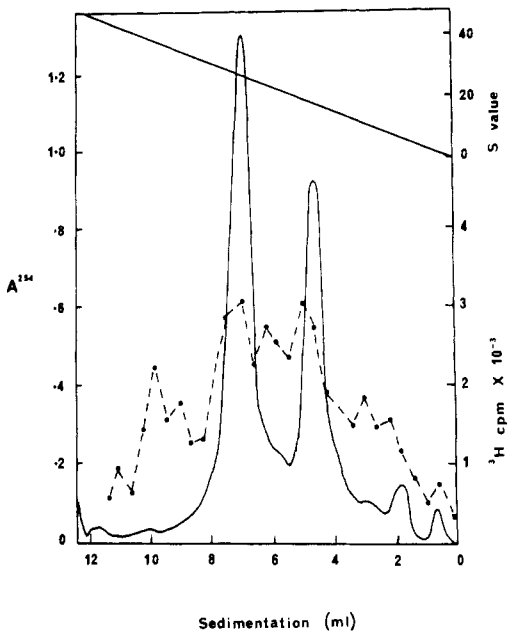

FIGURE 1: Erythroblasts were incubated with 100 μCi of [^{3}H]uridine for 50 min. Total RNA was isolated and centrifuged on 10–40% sucrose gradients in NET, at 30 000 rpm for 16 h at 4 °C in a Beckman SW41 rotor. Fractions (0.5 mL) were collected from the gradient and aliquots counted for ^{3}H radioactivity. A^{260} profile (—); ^{3}H cpm (●- - -●).

1–5% (w/v) in preequilibrated medium and shaken slowly at 39 °C. Incorporation was stopped by the addition of 20 volumes of ice-cold NKM, followed by immediate centrifuging (1000*g*, 3 min) to pellet cells.

Chicken Globin mRNA Preparation. Chicken globin mRNA was prepared as described by Crawford et al. (1977) and further purified by oligo(dT)-cellulose chromatography (Aviv & Leder, 1972).

Isolation of Total RNA from Erythroblasts. Packed erythroblasts (0.5 to 1.0 mL; about 5×10^9 cells), previously washed twice in ice-cold NKM, were resuspended by addition of 0.1–0.2 mL of NKM. The erythroblast suspension was added dropwise, with vortex mixing to 10 mL of cell lysis solution, consisting of phenol/chloroform (1:1) equilibrated with 10% (v/v) SET 9 (2% sodium dodecyl sulfate, 0.01 M EDTA, 0.02 M Tris-Cl, pH 9.0) and heparin, 1 mg/mL.

On completion of lysis, 10 mL of SET 9 was added. The mixture was shaken vigorously for 1 min and centrifuged (20 000*g*, 3 min) to separate the phases. The upper phase was separated by a Pasteur pipet and each phase reextracted. Nucleic acids were precipitated from the combined aqueous phases with ethanol and then dissolved in 0.5 mL of SET 7.4 (2% sodium dodecyl sulfate, 0.01 M EDTA, 0.02 M Tris-Cl, pH 7.4) and centrifuged on 5–20% linear sucrose gradients in one-half strength SET 7.4 over a 1-mL pad of 50% sucrose. Centrifugation was at 160 000*g* for 4 h in a Beckman SW41 rotor, at 17 °C. After centrifugation, 0.8 mL was carefully removed from the top of the gradient, and the remainder of the nucleic acids in the gradient (larger than 4–5S in size) were recovered by ethanol precipitation.

The nucleic acid precipitate containing erythroblast RNA and DNA was dissolved in 1 volume of 0.001 M EDTA, 0.01 M Tris-Cl (pH 7.4), and 3 volumes of 4.0 M sodium acetate, 0.005 EDTA (pH 6.0) was added (Kern, 1975). After standing the mixture at 0 °C for at least 5 h, precipitated RNA was pelleted by centrifugation (20 000*g*, 15 min). The supernatant, containing most of the DNA, was removed by aspiration. The RNA was washed once in 2.0 mL of 3.0 M sodium acetate, 0.004 M EDTA (pH 6.0), pelleted again by centrifugation, dissolved in 0.001 M EDTA, 0.01 M Tris-Cl (pH 7.4), and ethanol precipitated. Redissolved RNA was fractionated on 10–40% sucrose gradients in 0.01 M NaCl, 0.001 M EDTA, 0.01 M Tris-Cl. Prior to loading, erythroblast RNA solutions were heated at 65 °C for 10 min to ensure disaggregation of the RNA (Haines et al., 1974).

Globin cDNA Synthesis. Globin cDNA, labeled with [^{32}P]dCTP (20–40 Ci/mmol), was synthesized as previously described (Crawford et al., 1977) and used in hybridization reactions driven by chicken globin RNA.

Larger amounts of chicken globin cDNA were needed for cDNA driven hybridization reactions. Larger scale cDNA synthesis was similar to that previously described (Crawford et al., 1977) except that the cDNA was labeled with [^{32}P]-dCTP of specific activity 0.6 Ci/mol, at a concentration of 300 μM. The reaction volume of 500 μL, containing 50–80 μg of globin RNA purified by oligo(dT)-cellulose chromatography, 25 μg/mL oligo(dT)$_{12-18}$, and saturating amounts of AMV reverse transcriptase yielded 20–50 μg of globin cDNA.

RNA–cDNA Hybridizations. RNA driven hybridization reactions, containing 2000–10 000 cpm of cDNA, were carried out and assayed as previously described (Crawford et al., 1977).

Globin cDNA-excess reactions were used to probe erythroblast fractions for ^{3}H-labeled globin mRNA. Cr_0t analysis showed that the total globin RNA content of erythroblast RNA is about 0.5% (unpublished results). Assuming that all the globin RNA might be within any one fraction of the erythroblast RNA, each hybridization contained at least a two- to threefold excess of globin cDNA to globin mRNA. In this way, each hybridization contained sufficient globin cDNA to hybridize with all globin RNA sequences. Accordingly each reaction contained 0.5–1.0 μg of globin cDNA, and [^{3}H]RNA (containing a maximum of 0.2–0.4 μg of globin mRNA). Reaction mixtures containing the labeled RNA sample without globin cDNA were also prepared to determine the RNase resistant background dpm. Hybridizations of 60–80 μL were incubated at 60 °C for 2 h and assayed for [^{3}H]globin RNA as described by Ross (1976).

Results

Characterization of Purified Erythroblast RNA. Erythroblasts (dividing cells from the bone marrow of anemic chickens) and not reticulocytes (nondividing erythroid cells from the circulation (Williams, 1972)) were used as the source of RNA for this study. Erythroblasts are 50 to 100 times more active in RNA synthesis than reticulocytes (unpublished observations), and we considered that this greater activity would be an advantage in searching for a precursor in pulse-labeled RNA.

Even though any precursor to globin mRNA should be located within the nucleus of erythroblasts, the RNA isolation procedure used here did not involve the prior isolation of nuclei, because of the high levels of endogenous RNase (unpublished results) and consequent probability of RNA degradation during preparation of the nuclei. Despite this precaution, endogenous RNase activity during the initial stages of the RNA isolation procedure was still potentially a major factor in preventing the detection of a higher molecular weight precursor to polysomal globin mRNA. The intactness of isolated erythroblast RNA was judged by the optical density profile of 18S and 28S ribosomal RNAs, and by the presence of the 40–45S precursor to 18S and 28S ribosomal RNA.

As shown in Figure 1, both 18S and 28S ribosomal RNAs

from erythroblast RNA preparations sediment in sucrose gradients as major, discrete peaks. The length of 28S ribosomal RNA molecules is approximately twice that of 18S ribosomal RNA (Pinder et al., 1974), and therefore a ratio of A_{260} values of about 2:1 is expected in the absence of any breakdown. The distribution of A_{260} units in 28S and 18S RNA (Figure 1) shows such a ratio for isolated erythroblast RNA, suggesting that cytoplasmic RNA, at least, has not been degraded.

More importantly the erythroblast RNA contained undegraded nuclear RNA. In higher eukaryotes, 18S and 28S ribosomal cistrons are transcribed into a single 40–45S precursor RNA species which is then processed into mature 18S and 28S ribosomal RNA (Perry et al., 1970). The presence of intact 40–45S ribosomal precursor RNA within the erythroblast RNA preparation would suggest that nuclear RNA generally had not been degraded extensively during isolation. Hybridization experiments using labeled 18S and 28S ribosomal cDNAs detected a discrete 40–45S precursor ribosomal RNA species (data not shown). This rRNA-precursor region corresponded to the 40–45S region labeled with [^{3}H]uridine readily seen in Figure 1. It follows that globin mRNA precursor molecules should be present within the erythroblast RNA preparation.

The Size of Globin RNA Sequences in Chicken Erythroblast RNA. Two different hybridization approaches were used to probe for a higher molecular weight precursor to 10S polysomal globin mRNA. RNA-excess reactions, using trace amounts of highly labeled globin cDNA, were hybridized with erythroblast RNA fractions in the 20S to 50S size range, for a time sufficient to detect globin RNA sequences present at levels as low as one molecule per cell. Alternately, globin cDNA-driven hybridizations were used to probe specifically for labeled globin RNA sequences in the 10S to 20S size range.

1. *RNA-Excess Hybridization with Fractions of Erythroblast RNA.* The ability to detect RNA sequences present in low concentrations is one major advantage of this hybridization approach. Using 12S polysomal embryonic feather keratin mRNA (a gift of Mr. B. Powell), this approach was shown to be sufficiently sensitive to detect mRNA sequences present at the level of one to five molecules per cell.

Keratin mRNA (5–10 ng), an RNA sequence not expected to be normally present within avian erythroid cells, was added as a marker RNA to chicken erythroblasts immediately after lysis with phenol:chloroform. This is equivalent to one to five keratin mRNA molecules per erythroblast cell. Chicken erythroblast RNA was then purified and centrifuged on sucrose gradients. Keratin mRNA sequences sedimenting at 12 S were readily detected by hybridization with highly labeled [^{32}P]-keratin cDNA probe (data not shown). Hence this RNA isolation procedure and the RNA-excess hybridization approach enabled RNA sequences present at a level of one to five molecules per cell to be readily detected. In addition, keratin mRNA sequences were not located in regions larger than 12 S, demonstrating that keratin mRNA sequences at least did not aggregate during the work-up and fractionation procedures.

Despite the sensitivity of RNA-excess hybridizations, this approach was unable to detect any globin mRNA precursor larger than 20S. Total erythroblast RNA was centrifuged on a sucrose gradient, and RNA sedimenting at values greater than 20S was recentrifuged on a second sucrose gradient to enrich the higher molecular weight fractions for any putative globin RNA precursor, above the background of excess polysomal globin mRNA sedimenting at 10 S. Without the second centrifugation, there was sufficient polysomal globin mRNA

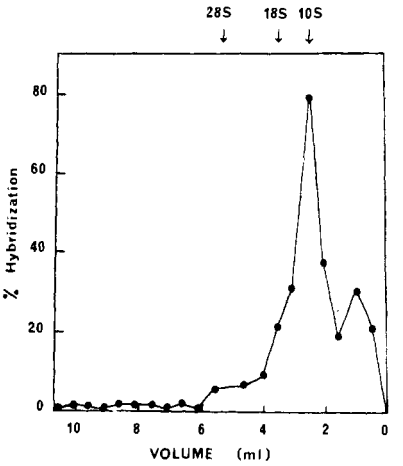

FIGURE 2: Total erythroblast RNA was sedimented on sucrose gradients and RNA in the 20–50S region of the gradient was collected and recentrifuged. Fractions (0.5 mL) were collected and hybridized with highly ^{32}P-labeled chicken globin cDNA for 120 h in a reaction volume of 50 μL.

present to mask the detection of low levels of any globin mRNA precursor. Fractions were collected from the second gradient, and the RNA from each fraction was hybridized to highly labeled globin cDNA. The hybridizations were incubated for a time sufficient to allow complete hybridization of the labeled cDNA, with any globin RNA sequences present at the level of one molecule per cell. As shown in Figure 2, no convincing evidence for globin RNA sequences between 20 S and 50 S in size could be established, whereas these sequences were readily seen in the 10S region of the second sucrose gradient. Hence the low levels of globin RNA sequences sedimenting in the 20–50S region of the first sucrose gradient were not demonstrably larger in molecular weight than polysomal globin mRNA.

The conclusion from repeated experiments of this type was that no globin mRNA precursor sedimenting from 20 S to 50 S could be detected at the one molecule per cell level within chicken erythroblast RNA. Accordingly, no further attempt was made to locate globin RNA sequences within this size range.

This approach was also used initially to probe for globin RNA sequences in the 10S and 20S size range. However, despite repeated centrifugation to enrich this size class of RNA for any globin RNA precursor above a background of 10S polysomal globin mRNA, no firm evidence for a larger precursor molecule was obtained. In each case, excess polysomal globin RNA masked the detection of low levels of any precursor that may have been present. Hence an alternative approach, using globin cDNA-driven hybridizations, was used to probe this size class of erythroblast RNA, for a precursor to polysomal globin mRNA.

2. *Globin cDNA-Excess Hybridization to Avian Erythroblast RNA Labelled in Vitro.* When erythroblasts are incubated with a short pulse of [^{3}H]uridine, the amount of [^{3}H]-RNA in hybrid form after hybridization of the erythroblast RNA with excess unlabeled globin cDNA is a relative measure of newly synthesized globin mRNA sequences. The vast majority of globin polysomal mRNA is already present in the cells prior to pulse labeling, and is therefore unlabeled, and not detected by this technique. In this way, the presence of low levels of newly synthesized RNA is not masked by a vast excess of steady-state RNA.

Erythroblasts were incubated with [^{3}H]uridine for 5 min, and the RNA isolated from these cells was sedimented on su-

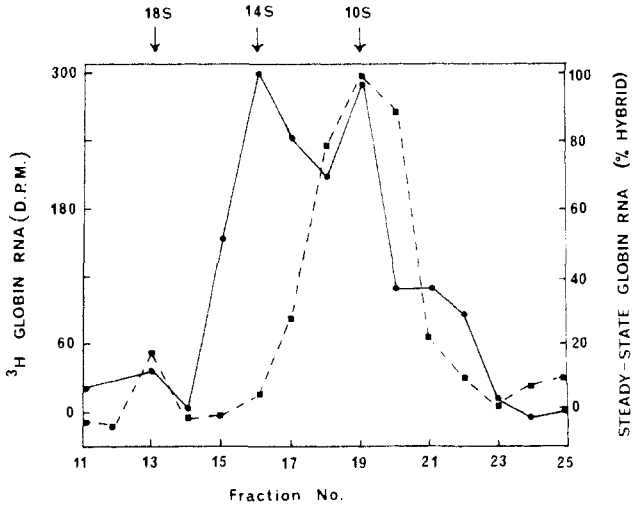

FIGURE 3: Chicken erythroblasts (0.25 mL packed cells) were pulse labeled with 4 mCi of [^{3}H]uridine in 4 mL of Eagles medium for 5 min. RNA was isolated from the washed cells and centrifuged on 10–40% sucrose gradients at 38 000 rpm for 16 h at 4 °C. Fractions (0.4 mL) were collected dropwise from the bottom of the gradient, and the 18S and 28S ribosomal RNA was located by measuring the optical density of each fraction. Fractions that contained RNA sedimenting between 5S and 22S were ethanol precipitated and hybridized separately to 0.5 μg of chicken globin cDNA in a 70-μL hybridization volume. Control hybridizations contained no chicken globin cDNA. Labeled RNA sequences hybridizing to globin cDNA were detected by RNase assay. Steady-state globin sequences were located by RNA-excess hybridization, and S1 nuclease assay as described in Methods. ^{3}H-labeled globin RNA (●—●); steady-state globin RNA (■- - -■).

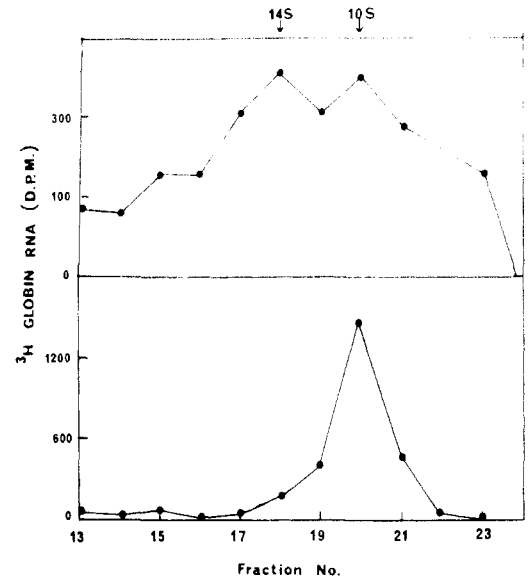

FIGURE 4: Erythroblasts (0.25 mL packed cells) were pulsed for 3 min with 4 mCi of [^{3}H]uridine. RNA was isolated immediately from half these cells. To the remainder of the cells, a 100-fold excess of unlabeled uridine was added and incubation carried on for a further 30 min prior to RNA isolation. Labeled globin RNA sequences were detected in both pulsed and pulse-chased RNA as described in Figure 5. (Top Panel) Pulse-labeled globin RNA; (bottom panel) pulse chased globin RNA.

crose gradients. Fractions of about 0.4 mL were collected, and the RNA from fractions sedimenting between 5 S and 22 S were hybridized separately with 0.5–1.0 μg of globin cDNA. Control incubations containing no globin cDNA were also carried out. The level of RNase resistant ^{3}H dpm in both the cDNA and control hybridizations was determined by digesting with RNase, under conditions in which RNA in hybrid form is undigested (Ross, 1976).

For each RNA fraction, the difference between these two assays indicates directly the number of dpm within globin RNA sequences. The difference between these assays is plotted in Figure 3, demonstrating the distribution of ^{3}H-labeled globin RNA sequences in the 5S to 22S region of the gradient.

After incubating erythroblasts for 5 min with [^{3}H]uridine, about 40–45% of the ^{3}H-labeled globin RNA sequences sedimented as a 14S species, while 60% sedimented at 10 S.

Separate hybridizations, using the same RNA fractions and high specific radioactivity [^{32}P]globin cDNA, were carried out to determine the distribution of steady-state globin mRNA within this gradient. As shown in Figure 3, the distribution of steady-state globin mRNA within this gradient is centered around 10 S.

Hence two types of hybridizations have demonstrated that two separate globin RNA types are present in erythroblast RNA after sucrose gradient centrifugation. One of these sediments at the same s value (10 S) as polysomal globin mRNA. The properties of the 14S globin RNA molecules partly satisfy the criteria for a higher molecular weight precursor to polysomal globin mRNA. Firstly, it sediments at a higher s value than polysomal globin mRNA, and secondly, it is a newly synthesized RNA species.

Although these experiments have detected globin mRNA sequences in two separate size classes of RNA, they have not shown that the globin RNA sequences in the 14S species are processed to 10S polysomal length globin mRNA. In other words, they have not demonstrated that the 14S species is a functional precursor to polysomal globin mRNA.

To determine whether the 14S globin RNA satisfies the criteria of a functional precursor, pulse-chase experiments were carried out. Erythroblasts were pulsed for 3 min with [^{3}H]-uridine, and RNA was isolated immediately from half the cells in the incubation. The [^{3}H]uridine in the remaining half of the incubation was diluted out with a 100-fold excess of unlabeled uridine, to minimize further incorporation of ^{3}H into erythroblast RNA. The erythroblasts in this half of the medium were incubated for a further 30 min, prior to RNA isolation.

RNA from both pulsed cells and pulse-chased cells was centrifuged on sucrose gradients, and separate size-class fractions were hybridized with excess unlabeled globin cDNA to locate ^{3}H-labeled globin RNA sequences. As shown in Figure 4, RNA isolated from pulsed cells contained about equal amounts of 14S and 10S labeled globin RNA, whereas the distribution of labeled globin RNA in pulse-chased RNA was centered around 10 S.

If the 14S globin RNA is a functional precursor to polysomal mRNA, and not simply turned over rapidly during the chase incubations, then the ^{3}H dpm in 14S globin RNA on the pulse gradient would sediment at 10 S after the chase period. In other words, the sum of the ^{3}H dpm in 14S globin RNA and 10S globin RNA on the pulse gradient would be equivalent to the number of ^{3}H dpm in 10S polysomal globin RNA after the chase incubation. The results in Figure 4 can be used to demonstrate that the numbers of ^{3}H dpm in globin RNA sequences in the pulse and pulse chase gradients are 2430 and 2508, respectively. Hence no net loss of ^{3}H dpm in globin RNA had occurred during the chase incubation. This result is consistent with the proposal that the 14S globin RNA species is a functional precursor to 10S polysomal globin mRNA.

As well as detecting a 14S precursor to polysomal globin mRNA, these experiments have enabled the half-life of the precursor to be estimated. After pulsing erythroblasts with

[^{3}H]uridine for 3 min, 50% to 60% of the labeled globin RNA sedimented at 14S (Figure 4), whereas, after 5 min, about 40% was detected in the 14S species (Figure 3). Hence the half-life of the precursor is about 3 to 4 min. There is additional evidence to support this estimate of the half-life. When erythroblasts were pulsed for 20 min with [^{3}H]uridine, then only 12% of the labeled globin RNA sequences sedimented as a 14S species (data not shown).

This estimate of 3 to 4 min for the half-life of the 14S globin RNA precursor is the maximum possible time taken for half the precursor population to be processed into 10S globin RNA since it makes no allowance for the time taken for [^{3}H]uridine uptake into erythroblasts, and its conversion to [^{3}H]UTP.

Oligo(dT)-Cellulose Chromatography of Erythroblast Globin RNA. To determine whether the 14S globin mRNA precursor is polyadenylated, erythroblast RNA fractions were passaged through oligo(dT)-cellulose. Erythroblasts were incubated with [^{3}H]uridine, and total RNA was isolated and centrifuged on sucrose gradients. Fractions (0.4 mL) were collected and RNA fractions sedimenting between 5S and 20S were passaged separately through oligo(dT)-cellulose under conditions in which polyadenylated RNA binds to oligo(dT). Bound RNA was eluted as described by Aviv & Leder (1972). Globin cDNA-excess hybridizations were used to probe for labeled globin RNA sequences in the bound and unbound fractions of each RNA size class.

As shown in Figure 5, labeled 14S globin RNA was detected in the bound RNA fraction only; no significant level of labeled 14S globin RNA was detected in the unbound fraction. Hence the ability of oligo(dT)-cellulose to bind labeled 14S globin RNA sequences demonstrates that the 14S precursor to polysomal globin mRNA is polyadenylated.

It is noted that about 20% of the labeled 10S globin RNA appeared in the unbound RNA fraction (Figure 5). In our hands this is equivalent to the proportion of 10S globin RNA appearing in the unbound fraction when steady-state polysomal globin mRNA is passaged through oligo(dT)-cellulose.

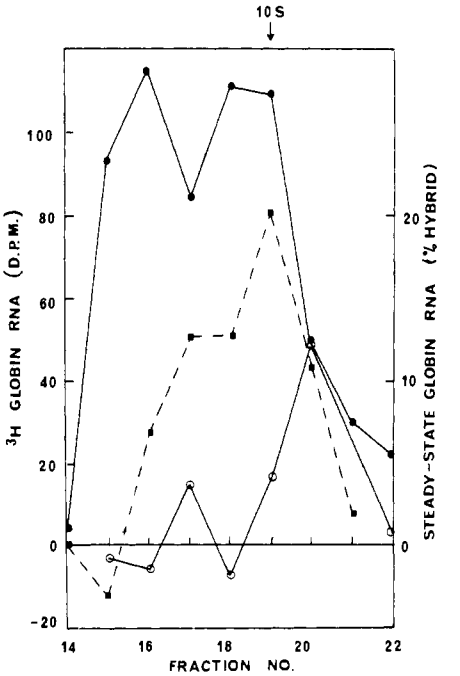

FIGURE 5: Erythroblasts were labeled with [^{3}H]uridine as described in Figure 3, and total RNA was isolated and centrifuged on 10–40% sucrose gradients. Fractions (0.4 mL) were collected and RNA fractions sedimenting between 5S and 20S were passaged separately through oligo(dT)-cellulose as described by Aviv & Leder (1972). Bound and unbound RNA was collected from each fraction. Labeled (newly synthesized) globin RNA sequences and steady-state globin RNA sequences were detected by cDNA-excess hybridizations and globin RNA-excess reactions, respectively, as described in Methods. ^{3}H-labeled globin RNA bound to oligo(dT)-cellulose (●—●); ^{3}H-labeled globin RNA unbound to oligo(dT)-cellulose (○—○); steady-state globin RNA within "bound" RNA (■- - -■).

Discussion

The experiments described here were aimed at investigating the properties of newly synthesized globin RNA sequences in chicken erythroblasts. Firstly, total erythroblast RNA was isolated, and the precursor to 18S and 28S ribosomal RNA was detected as a discrete 40–45S RNA species, indicating that the method used for RNA preparation enabled the isolation of undegraded nuclear RNA.

Hybridizations driven by globin cDNA detected a precursor to globin mRNA, sedimenting as a 14S RNA species in aqueous sucrose gradients. The properties of this 14S species satisfied three criteria of a larger precursor to polysomal globin mRNA: (1) it is newly synthesized; (2) it is higher in molecular weight; (3) the globin coding nucleotides in the 14S species can be chased into 10S globin mRNA.

Two further properties of this precursor were determined. Firstly, its half-life is no longer than 3 to 4 min. In comparison, the half-life of the 14S precursor to mouse fetal globin mRNA is about 45 min (Ross, 1976) and that of the 15S precursor found in Friend erythroleukemic cells induced with dimethyl sulfoxide appears to be about 8 to 10 min (Curtis & Weissman, 1976). Hence the processing time of the 14S chicken globin RNA precursor is faster than that for both these precursors to mouse globin mRNAs. Furthermore, its processing time is much shorter than the average half-life of HnRNA generally occurring within avian erythroid cells, estimated to be about 18 min (Williamson & Tobin, 1977). Secondly, the 14S globin RNA precursor binds to oligo(dT)-cellulose, under conditions where nonpolyadenylated RNA is not bound. Hence the precursor to chicken polysomal globin mRNA is polyadenylated.

The size of this precursor is approximately equivalent to the discrete globin mRNA precursors found in mouse erythroid cells (Ross, 1976; Curtis & Weissman, 1976) and to the nuclear globin RNA species detected in duck erythroid cells (Macnaughton et al., 1974). The precursor to chicken globin mRNA sediments at 14 S in aqueous sucrose gradients, indicating that it is about twice as large as the polysomal globin mRNA.

It is unlikely that the chicken globin RNA sediments at 14 S because of aggregation. Firstly, the steady-state 10S polysomal globin mRNA, which serves as an ideal internal control, shows no evidence for aggregation (Figure 3). Secondly, no 14S globin RNA species was detected after a 30-min chase experiment (Figure 4). Thirdly, heat treatment of the RNA sample prior to sucrose gradient centrifugation is sufficient to disrupt RNA aggregates (Haines et al., 1974). On these bases, the 14S value of this chicken globin mRNA reflects its true molecular integrity and is not a result of aggregation.

Despite differences in approach, the precursor detected in this work is probably a similar RNA species to that detected in duck erythroid nuclear RNA by Macnaughton et al. (1974). However, the work described here demonstrates that the globin RNA sequences in the newly synthesized chicken 14S species are processed to 10S polysomal length globin mRNA. In other words, these experiments indicate that the 14S species is a functional precursor to polysomal globin mRNA. Furthermore, we show that the maximum half-life of the precursor is

3 to 4 min and have confirmed that the precursor is polyadenylated. Whereas Macnaughton et al. (1974) isolated nuclear RNA from duck erythroid cells obtained from the circulation, the work described here used total RNA (nuclear RNA + cytoplasmic RNA) from dividing erythroid cells isolated from the bone marrow of anemic chickens.

It is not possible to say whether the 14S globin RNA species is the primary transcript of the globin genes. In contrast to other reports (Imaizumi et al., 1973; Spohr et al., 1976; Williamson & Tobin, 1977) experiments described here failed to detect any globin RNA sequences larger than 14 S. From the dpm detected in 14S globin RNA, and assuming there is no uridine pool within erythroblasts, it is possible to estimate that there is at least one precursor molecule per five cells. In fact, the uridine pool within erythroblasts is considerable (unpublished results) so that this estimate is a considerable underestimate for the number of precursor molecules per cell. On this basis, the RNA-excess hybridizations carried out on the erythroblast RNA sedimenting between 20S and 50S should have detected globin sequences within this size range, if they were present. If the globin genes are transcribed into a product larger than 14S, then the half-life of this RNA species must be extremely short.

Between 10% (Spohr et al., 1976) and 30% (Williamson & Tobin, 1977) of chicken erythroid cell HnRNA is polyadenylated. Oligo(dT)-cellulose chromatography has demonstrated that the 14S chicken globin precursor is within this 10–30% of polyadenylated HnRNA. It is unlikely (but unproven) that the nucleotides in the 14S precursor which are not within 10S polysomal globin mRNA are all adenylic acid residues since the poly(A) region of the precursor would then be about 600 residues in length.

Because the precursor is polyadenylated, it is tempting to suggest that the polysomal globin mRNA sequences are adjacent to the poly(A) at the 3′ end of the molecule. However, the possibility that the globin mRNA sequences are located near the 5′ end of the precursor cannot be excluded. In this latter case, the polysomal globin mRNA sequences would then be cleaved from the 3′ sequences (including the poly(A)) of the precursor, and be polyadenylated directly at a later stage in the processing.[2]

[2] Since this manuscript was submitted for publication, two other pertinent manuscripts have appeared. These report 27S and 14–15S globin mRNA precursors in dimethyl sulfoxide induced Friend erythroleukemic cells (Bastos & Aviv, 1977) and in erythroid cells from the circulation of anemic ducks (Strair et al., 1977). In the second case, the half-life of 14S globin precursor in duck erythroid cells is much greater than the half-life of the 14S precursor reported here. It is noted that the duck globin precursors were detected in erythroid cells from the circulation, whereas the 14S globin precursor reported here was detected in RNA from dividing erythroid bone marrow cells of anemic chickens. From the comparison of half-lives of the 14S precursors detected in anemic duck circulatory erythroid cells (Strair et al., 1977) and in chicken bone marrow cells reported here, it is likely that the half-life of the 14S precursor is much shorter in very early avian erythroid cells. In other words, as the erythroid cells mature, the synthesis and processing of the 14S globin precursor take progressively longer. It is apparent that the half-life of the 27S globin RNA precursor is much less than that of the 14S precursor (Strair et al., 1977). Hence the 27S precursor was probably not detected in chicken bone marrow cells, because of its extremely short half-life.

Acknowledgments

We gratefully acknowledge the gift of AMV reverse transcriptase supplied by the Office of Program Resources and Logistics, Viral Cancer Program, Viral Oncology, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. 20014. We thank Dr. R. H. Symons for the gift of [α-^{32}P]dCTP, Mr. B. Powell for supplying chicken keratin mRNA, and Mrs. J. Dinan for competent assistance.

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