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Demonstration of Globin Messenger Sequences in Giant Nuclear Precursors of Messenger RNA of Avian Erythroblasts

(precursor-mRNA/hemoglobin/RNA-directed DNA polymerase/anti-messenger DNA/hybridization)

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ABSTRACT **Highly purified globin mRNA from ducks was copied with RNA-directed DNA polymerase from avian myeloblastosis virus into anti-messenger DNA. With excess RNA, more than 90% of this DNA annealed back to its template with a $C_0t/2$ value of 7.5×10^{-4} mol·sec·liter^{-1}; the melting temperature of the hybrid was 86°. Giant nuclear RNA fractions with sedimentation coefficients of more than 50 S formed hybrids of almost equal stability at $C_0t/2$ values of 0.05–0.42 mol·sec·liter^{-1}, indicating amRNA content of 0.3–1.5%. 12S RNA from the same polyribosomes and nuclear giant RNA from HeLa cells did not cross-hybridize. Although a large part of the giant RNA broke down in 99% dimethylsulfoxide gradients, RNA fractions sedimenting faster than 28S rRNA still were found to consist of up to 0.03% globin mRNA sequences. Thus, the mRNA sequences are contained in the covalent structure of giant nuclear precursors, which are termed precursor-mRNA.**

Since the first observation of giant messenger-like RNA with a molecular weight of more than 3.5×10^6 (mlRNA) (1–5) in the nuclei of animal cells, the function of this class of RNA has been questioned. Indirect evidence first led us to postulate that this RNA was a precursor to mRNA (3, 4, 6), in analogy to the nucleolar precursor of ribosomal RNA (rRNA) (1, 7, 8). High C_0t hybridization–competition experiments demonstrated base-sequence homology between polyribosomal mRNA and giant nascent mlRNA from HeLa cells (9). Viral-specific RNA in SV40-transformed (10) and polyoma-infected (11) cells are transcribed as giant RNA molecules several times the size of the viral genome.

We report here direct evidence that giant nascent mlRNA does indeed contain the same nucleotide sequence as functional mRNA. Globin mRNA from ducks was transcribed by RNA-directed DNA polymerase (12,13) from oncornaviruses into anti-messenger DNA (amDNA). This DNA could be annealed with giant nascent nuclear RNA, forming a hybrid almost as stable as that with the 9S mRNA itself.

METHODS

9S and 12S Polyribosomal lmRNA Was Prepared from immature duck erythrocytes as described (14), followed by hot phenol extraction, DNase treatment (5 μg/ml, 30 min, 0°), and elimination of DNase by Macaloid adsorption. The 9S preparations thus obtained directed the synthesis of all duck globins in heterologous cell-free ribosomal systems (14, 15) and in *Xenopus* oocytes (16).

Nuclear pre-mRNA. Duck erythroblasts were labeled with $^{32}PO_4$ for 2–3 hr. Nuclei were isolated and RNA was extracted by hot phenol (17) and was fractionated by zonal sucrose gradient centrifugation. DNA was eliminated by DNase treatment first of the nuclei and then of the isolated zonal gradient fractions. Sedimentation constants were estimated by recentrifugation of the zonal fractions with a 45S precursor-rRNA marker on sucrose gradients in an SW56 rotor of a Spinco ultracentrifuge.

amDNA Complementary to 9S mRNA. In order to quantitate hybridization of amDNA, reaction mixtures were digested with DNase S_1 from *Aspergillus oryzae*, which does not hydrolyze double-stranded DNA or RNA–DNA hybrids (18). RNA samples in 0.3 M NaCl–50 mM Tris·HCl (pH 7.4)–0.1% Na dodecyl sulfate were denatured (4 min, 100°) and then annealed with 5 or 10 pg (500–1000 cpm) of [^{3}H]dG- and [^{3}H]dC-labeled globin amDNA at 65° (19) for 2 min to 2 days in 10-μl volumes. Nuclear DNA was hybridized in 5 μl for up to 2 days. Hybrid formation was assayed by rinsing the incubation mixture into 200 μl of 30 mM Na-acetate (pH 4.5), 0.15 M NaCl, 1 mM $ZnSO_4$, and 5 μg of denatured salmon-sperm DNA, containing 2 units/ml of partially purified DNase S_1 (18). Incubation was for 90 min at 37°, followed by trichloroacetic acid precipitation and assay for radioactivity. The background was determined in parallel by digestion of self-annealed amDNA.

RESULTS AND DISCUSSION

Synthesis of amDNA Complementary to 9S Globin mRNA. Eukaryotic mRNA can serve as a template for RNA-directed DNA polymerase of oncornaviruses (19–21). With purified polymerase from avian myeloblastosis virus (AMV) (22) the synthesis of complementary DNA depended on the addition of RNA and on oligo(dT) (approximate chain length, 20 bases) as a primer (19–21). The amount of DNA synthesized and the size of the product depended on the concentration of the deoxyribonucleoside triphosphates present in the reaction mixture (Fig. 1). With relatively low concentrations (40–50 μM) of labeled triphosphates, the enzyme synthesized only 0.06 μg of DNA per μg of mRNA added to the reaction, and the product sedimented at about 5–7 S on an alkaline sucrose gradient. However, when the same labeled triphosphates were present at 3.5–4.0 mM, 0.5 μg of DNA per μg of mRNA was synthesized, and its size increased to an average of 6.9 S, corresponding to a molecular weight of 1.5×10^5 (23). The largest molecules synthesized under these

Abbreviations: pre-mRNA, precursor to messenger RNA [replaces the largely synonymous mlRNA (6), HnRNA (5), and D-RNA (1, 2)]; rRNA, ribosomal RNA; pre-rRNA, precursor to rRNA; amDNA, DNA strand copied from mRNA by the RNA-directed DNA polymerase (anti-messenger DNA); Hb, hemoglobin; AMV, avian myeloblastosis virus; Tm, melting temperature.

conditions could correspond to the full length of the template mRNA used. For the hybridization experiments reported in this paper, 5–7S amDNA was used exclusively.

Hybridization of amDNA with mRNA and mlRNA. Extent and kinetics of back-hybridization, as well as the melting temperature of the hybrid, confirmed that the amDNA had been copied from the mRNA with fidelity. Fig. 3 shows that at least 90% of the amDNA synthesized by two preparations of RNA-directed DNA polymerase and from several preparations of mRNA formed nuclease-resistant hybrids. The melting profile of one of these hybrids is illustrated in Fig. 2. The sharp transition at 86° approximates that expected for a perfect DNA–RNA duplex of 52% G+C content (24). With

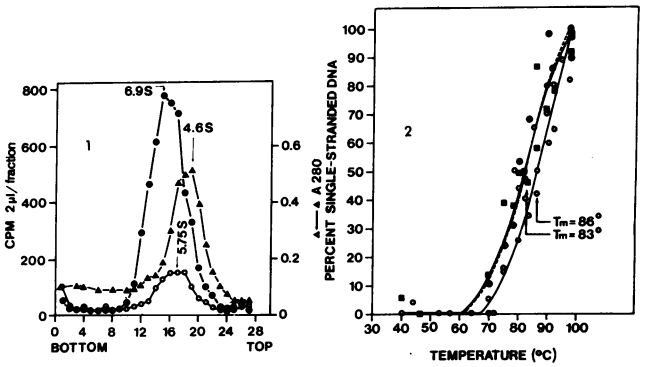

FIG. 1. (*left*). *Alkaline sucrose gradient sedimentation profile of globin amDNA.* Oligo(dT) primer was prepared by incubating poly(dT) (60 μg/ml) with pancreatic DNase (20 μg/ml, 30 min, 37°) in 10 mM Mg^{++}. Hb-mRNA (10 μg/ml) was added to a 20-μl reaction mixture containing: 50 μg/ml of purified RNA-dependent DNA polymerase from AMV (22), 2 μg/ml of oligo-(dT), 1 mM unlabeled and the indicated concentration of ^{3}H-labeled deoxy-ribonucleoside triphosphates, 10 mM $MgCl_2$, 10 mM KCl, 50 mM Tris·HCl (pH 8.3), 1.5 mM 2-mercaptoethanol, and 35 μg/ml of actinomycin D to prevent synthesis of double-stranded RNA (19). After incubation for 30 min at 37°, the reaction was stopped by addition of Na dodecyl sulfate to 1% and EDTA to 10 mM. The product was passed over Sephadex G-50 to remove unincorporated deoxyribonucleoside triphosphates. The excluded product was adjusted to 0.2 N NaOH, incubated for 20 min at 80°, and layered on a 5-ml alkaline sucrose gradient (5–20% linear sucrose gradient in 0.2 N NaOH–100 mM NaCl–10 mM EDTA; Spinco SW50.1, 45,000 rpm, 14 hr, 4°). Aliquots of each fraction were precipitated with 6% Cl_3CCOOH and counted on glass-fiber filters. For recovery of the amDNA, the peak fractions were pooled, neutralized, and precipitated at −20° with 2 volumes of ethanol in the presence of 15 μg of T4DNA. (O——O) Low triphosphate concentration (41.6 μM [^{3}H]dCTP, 12 Ci/mol; 51 μm [^{3}H]dGTP, 9.8 Ci/mmol). (●——●) High triphosphate concentration (3.47 mM [^{3}H]dCTP, 1.2 Ci/mmol; 40 mM [^{3}H]dGTP, 0.98 Ci/mmol). (▲——▲) Bovine-serum albumin run in a parallel neutral sucrose gradient.

FIG. 2. (*right*). *Tm of amDNA hybridized with globin mRNA and pre-mRNA.* Hb mRNA (9 S) (O– – –O) and two independent preparations of duck pre-mRNA, 30–100 S (●– – –●) and 50–70 S (■——■), were hybridized with amDNA at low concentration ($C_0t/2$ reached in about 10 hr) to minimize back-hybridization after melting. To determine the Tm of the hybrids formed, individual capillaries were heated to the temperature indicated on the abscissa for 2–3 min in a temperature-controlled waterbath. Their content was immediately rinsed into nuclease S_1 incubation mixtures and digested to determine the remaining hybrid.

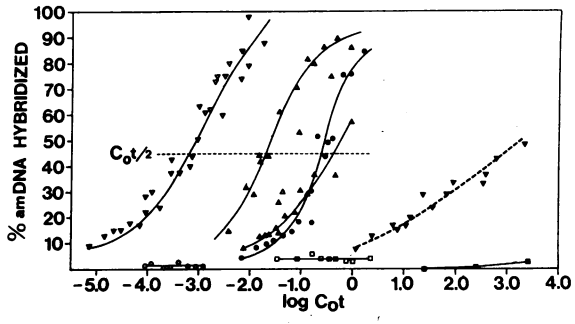

FIG. 3. *Hybridization kinetics of amDNA with various Hb-related RNAs or control RNA fractions and with nuclear DNA.* Where not specified, annealing was done in 10 μl with 5–10 pg/μl of labeled amDNA (500–1000 cpm) for 2 min to 2 hr. Individual RNA and DNA fractions were from duck erythroblasts if not specified. Hb mRNA (9 S): 0.01–1 ng/μl (▽——▽); polyribosomal 12S RNA: 0.1 ng/μl (O——O); pre-mRNA: 0.01–1.0 μg/μl, >80S (●——●) 50–80 S (▲— ▲), and 30–50 S (△——△); HeLa cell pre-mRNA (30–100 S): 1.0 μg/μl (□— —□); *E. coli* tRNA: 30 μg/μl (■—■); nuclear DNA (sheared, 5–7 S in alkali): 10 μg/μl in 5 μl for up to 48 hr (▼——▼). The percentage of hybridized amDNA is plotted according to Britten and Kohne (34).

excess RNA, annealing of globin amDNA to giant mlRNA of various sizes (30–100 S) led to hybrids of similar stability as that obtained by back-hybridization to the globin mRNA. Again, up to 90% of the amDNA hybridized (Figs. 3 and 5*B*). The Tm of the duplex was 83° (Fig. 2), close to that of the amDNA–mRNA duplex, indicating no more than 1–2% mismatched bases (25).

Several controls (Fig. 3) ruled out that nonspecific reactions could account for the hybridization observed. *Escherichia coli* tRNA did not anneal, indicating that there was no effect of RNA on the apparent resistance of the amDNA to DNase S_1. Giant mlRNA from HeLa cell nuclei did not anneal (up to a log C_0t of 0.5), indicating that hybridization of the amDNA was not due to nonspecific homologous sequences that might occur in giant mlRNA of animal cells in general. Finally, amDNA complementary to globin mRNA did not form hybrids with the second predominant mRNA species (12 S) from duck erythroblasts (26). This result argues that the annealing of amDNA to mlRNA does not reflect some sequence common to all messengers; e.g., most mRNAs, as well as mlRNA, contain oligo(A) (4, 27) and poly(A) sequences (28, 29). To insure that these particular nonspecific sequences did not lead to erroneous conclusions, we labeled the amDNA only with dC and dG. The absence of annealing with 12S RNA further implies that 12S RNA cannot be a precursor to globin mRNA (as has been suggested; ref. 30), and that our 9S and12S RNAs were not mutually contaminated. We thus conclude that giant nuclear RNA from avian erythroblasts contains the sequence of this cell's globin mRNA. These RNA molecules thus represent informational precursors to polyribosomal mRNA. Hence the identification of mlRNA as a precursor of messenger RNA (pre-mRNA) is justified.

The high melting temperature of the amDNA–pre-mRNA hybrid, as well as the complete annealing of the amDNA, appears to exclude the extensive occurrence in pre-mRNA of globin-related sequences. Grossly altered sequences with enough similarity to allow some hybrid formation should

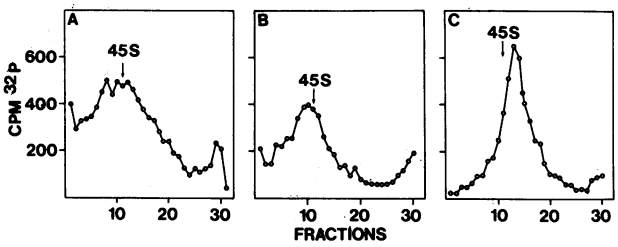

FIG. 4. *Characterization of pre-mRNA by sucrose gradient sedimentation.* pre-mRNA from duck erythroblasts was extracted and fractionated in a zonal rotor (see *Methods*). The sedimentation constants of the chosen fractions (*A*, *B*, *C*) were estimated, relative to a 45S pre-rRNA marker, by recentrifugation on isokinetic gradients (5–20%) sucrose in 10 mM triethanolamine–NaCl (pH 7.4)–10 mM EDTA–0.2% Na dodecyl sulfate–deoxycholate (Spinco SW56 rotor, 56,000 rpm, 90 min, 2°).

lead to a considerable lowering of the Tm of the hybrid or to a partial susceptibility to DNase S_1 of the annealed amDNA. A recent model of the chromosome (31) implies such nonfunctional sequences that have diverged in evolution from the globin mRNA gene actually used by the cell.

Quantitative Determination of Globin mRNA Sequences. The annealing of amDNA with globin mRNA and pre-mRNA represents a simple system of hybridization according to the definitions of Bishop (32). The active rate-determining reagent is a highly purified RNA of low base-sequence complexity; the concentration of globin mRNA sequences in the pre-mRNAs tested always exceeded that of amDNA by a factor of 10–100, so that the concentration of mRNA remained practically constant throughout the reaction. Since the rate of RNA–DNA hybridization is independent of the size of the RNA (33), this rate depended exclusively, at a constant amDNA input, on the concentration of globin sequences in a given RNA species.

Avian-erythroblast polyribosomes synthesize *in vivo* two hemoglobins, HbA and HbD, in a ratio of 4:1. Both contain two identical β chains; HbA contains in addition two α^A-chains, HbD two α^D-chains. The ratio of α^A to α^D to β is thus 4:1:5. The two α chains are very similar in aminoacid sequence; the β chain is different (Brown, L. G. & Ingram, V. M., personal communication). Therefore we assume that the base-sequence complexity* of the population of globin mRNA is 4×10^5, or twice the average molecular weight of a single 9S RNA molecule.

In order to quantitate the globin mRNA sequences in various RNA preparations, we analyzed the hybridization kinetics by drawing C_0t plots as proposed by Britten and Kohne (34). The value of the midpoint of the reaction ($C_0t/2$) characterized each individual RNA. Since the reaction regularly went 90% to completion, $C_0t/2$ values were taken at 45%. The amDNA–RNA reaction with several preparations of globin mRNA and of amDNA gave an average $C_0t/2$ value of 7.5×10^{-4} mol·sec·liter^{-1} (Fig. 3 and Table 1). Although no kinetic standards (33) valid for our conditions are available, this value is in the range expected (34, 35). Using this $C_0t/2$ value to represent 100% globin sequence, we determined that the percentage of globin message in three pre-mRNA fractions (isolated from a zonal gradient; Fig. 4) with molecular weights about 20, 5, and 2×10^6 were 0.30, 0.18, and 1.5%, respectively (Fig. 3 and Table 1). In the experiment of Fig. 5 and Table 3, two pre-mRNA fractions with molecular weights of more than 5×10^6 contained about 0.6% globin mRNA sequence.

Assuming the correctness of the molecular weights of the pre-mRNA and assuming that no RNA contains more than one globin sequence, we calculated that 10–50% (Table 1) of giant pre-mRNA molecules contain a globin sequence. As

*Base sequence complexity is the total molecular weight of nonidentical sequences in a nucleic acid preparation (32, 33). In our case it means total nucleotide sequence in an RNA preparation containing one complement of Hb-mRNA sequences (α and β chains).

TABLE 1. *Hybridization of globin amDNA with globin mRNA and pre-mRNA*

	9S mRNA	pre-mRNA fractions A	B	C
$C_0t/2$	7.5×10^{-4}	2.5×10^{-1}	4.2×10^{-1}	5.0×10^{-2}
% Hb mRNA	100	0.30	0.18	1.5
Experimental base sequence complexity	*4.0×10^5	1.3×10^8	2.2×10^8	2.7×10^7
Average molecular weight by sedimentation	*2.0×10^5	3.5×10^7	7.5×10^6	4.0×10^6
% of molecules with one Hb mRNA sequence	100	52	7	30

* Standards. $C_0t/2$ values were taken from Fig. 3. With the value obtained with 9S mRNA as kinetic standard, the fraction of pre-mRNA consisting of globin sequences and the base-sequence complexities were calculated (assuming a cumulative kinetic complexity of 4×10^5 for the α and β chains of Hb). The average molecular weights of pre-mRNA fractions were estimated from their apparent sedimentation rates; by dividing these values by one-half the base sequence complexity, we estimate the fraction of pre-mRNA molecules containing one globin chain.

TABLE 2. *Characteristics of pre-mRNA fractions isolated from $(CH_3)_2SO$ gradients*

	A			B		
	I	II	III	I	II	III
Estimated sedimentation constants	>28 S	20–28 S	<20 S	>28 S	20–28 S	<20 S
A_{260} measured	0.147	0.114	1.100	0.480	0.200	7.000
260/280 nm ratio	1.93	1.87	2.10	1.98	2.30	1.84
% A_{260} in pool	6	5	89	6	3	91
% cpm in pool	25	44	31	41	37	22

Pre-mRNAs are fractions from sucrose gradients (see Fig. 4*A*,*B*). Gradient pools *I*, *II*, and *III* are from $(CH_3)_2SO$ centrifugation (see Fig. 5*A*, *A'*).

TABLE 3. *Hybridization of globin amDNA with pre-mRNA and with subfractions from a $(CH_3)_2SO$ gradient*

	A				B			
	Input RNA	I	II	III	Input RNA	I	II	III
$C_0t/2$	0.13	2.37	0.71	0.12	0.14	7.94	1.00	0.32
% Globin mRNA	0.59	0.03	0.11	0.63	0.53	0.01	0.08	0.24
Base sequence complexity	3.4×10^7	6.3×10^8	1.9×10^8	3.2×10^8	3.8×10^7	2.2×10^9	2.7×10^8	8.4×10^7

$C_0t/2$ values were taken from Fig. 5*B'* and from similar experiments with the RNA fractions of Fig. 5*A'* [$(CH_3)_2SO$ gradient pools *I*, *II*, and *III*]. Percentage of globin sequence in pre-mRNA and base sequence complexities were calculated as explained in text and legend to Table 1.

shown below, the first assumption is only partially valid and no proof exists for the second. Possibly some pre-mRNA molecules contain several globin mRNA sequences; this would require several globin genes to exist in tandem on the DNA. Such an alternative seems unlikely since some point mutations affect the whole population of a given globin chain. However, models of DNA replication have been formulated that could account for multiplication of genes without evolutionary divergence (36).

Stability of Large Globin-Specific pre-mRNA in $(CH_3)_2SO$. Although pre-mRNA was isolated under conditions known to avoid aggregation, and although no trapping of labeled 9S mRNA by pre-mRNA upon cosedimentation was observed, we sought more rigorous controls to prove that the globin mRNA sequences are part of the covalent structure of giant pre-mRNA molecules. First, to exclude the possibility that some globin mRNA was hydrogen bonded to antimessenger sequences in giant RNA, we hybridized amDNA to pre-mRNA before and after heat denaturation of the RNA. Fig. 5*B* shows that both RNAs annealed with the same kinetics, demonstrating that in the pre-mRNA there are no antimessenger sequences competing with the amDNA. Second, several preparations of giant pre-mRNA (isolated from zonal sucrose gradients) were resedimented on 99% $(CH_3)_2SO$ gradients. $(CH_3)_2SO$ suppresses all hydrogen bonding in nucleic acids (37). Enough labeled pre-mRNA was centrifuged on $(CH_3)_2SO$ to allow determination in the pooled RNA fractions of concentration and purity (260/280-nm absorbancy ratio) before hybridization with globin amDNA (Fig. 5*A,A'*; Table 2). These experiments (Fig. 5; Table 3) gave the unambiguous answer that pre-mRNA fractions sedimenting in $(CH_3)_2SO$ more rapidly than 28S rRNA still hybridize with Hb-amDNA (Fig. 5*B'*). Thus, we conclude that the globin mRNA sequence is contained in the covalent structure of pre-mRNA molecules considerably longer than 28S rRNA [in $(CH_3)_2SO$].

Apparently only a very small proportion ($1\text{–}3 \times 10^{-4}$) of the pre-mRNA sedimenting fastest in $(CH_3)_2SO$ represents globin mRNA sequences. Three globin cistrons (molecular weight 6×10^5) correspond to 7×10^{-7} of the duck DNA (molecular weight of haploid genome DNA; 8×10^{11}). Since in erythroblasts about 5% of the duck DNA is transcribed (6), a population of RNA molecules with one pre-mRNA per transcribed gene should contain 1.4×10^{-5} of the RNA as globin sequences. Thus, since our data are consistent with this figure we must assume that, in steady state, only a small pool of nascent HnRNA molecules exists. In view of the rapid turnover of the largest, nascent pre-mRNA molecules, this figure is not unreasonable.

Although a fraction of the pre-mRNA on the $(CH_3)_2SO$ gradients still appeared large and contained globin sequences, a large proportion of the pre-mRNA (as measured by A_{260}) broke down in $(CH_3)_2SO$ to molecules sedimenting more slowly than 18 S. The molecules remaining in the faster-sedimenting zones (Fig. 5*A,A'*; Tables 2 and 3) showed a

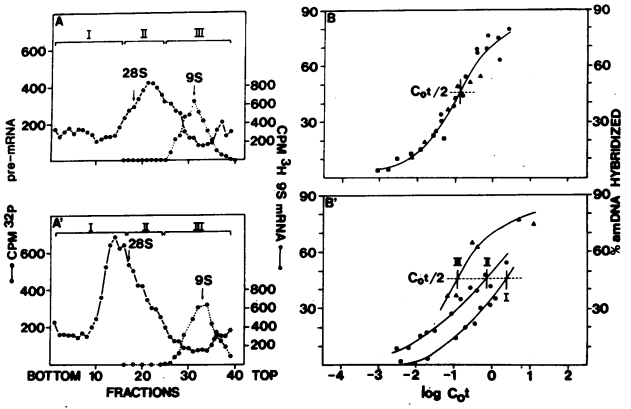

FIG. 5. *Determination of globin mRNA in pre-mRNA reisolated from $(CH_3)_2SO$ gradients.* (A) *$(CH_3)_2SO$ gradient centrifugation of pre-mRNA.* Two pre-mRNAs (preparations *A* and *B* of Fig. 4, different from those in Fig. 3) with approximate average sedimentation coefficients of 50 S (*A*) and 40 S (*A'*) were recentrifuged in 10-μg fractions in five parallel gradients containing 99% $(CH_3)_2SO$, 0–10% sucrose, and 1 mM EDTA (pH 7.0) (11) (Spinco SW56 rotor, 48,000 rpm, 27°, 4.5 hr). 28S [^{14}C]rRNA from HeLa cells and globin 9S [^{3}H]mRNA were centrifuged as markers in a parallel gradient. The radioactivity profiles were determined. Individual fractions were divided into pools *I*, *II*, and *III* as indicated, precipitated by 3 volumes of ethanol in 0.2 M NaCl, and sedimented (60–90 min, 16,000 × *g*). After suspension in 150 μl of 5 mM Tris–NaCl (pH 7.4), A_{260}/A_{280} ratios were determined (see Table 2). (B) *Hybridization of pre-mRNA before and after $(CH_3)_2SO$ centrifugation.* Pre-mRNA preparations *A* and *A'*, before or after fractionation in $(CH_3)_2SO$ gradients and reisolation as described above, were hybridized with [^{3}H]amDNA (see legend of Fig. 3). The individual fractions are characterized in Table 2 and 3. Their concentrations during annealing varied from 1 to 100 ng/μl. (*B*) Sample *A* before $(CH_3)_2SO$ fractionation; in some controls (○——○) denaturation of pre-mRNA before hybridization was omitted. (*B'*) Subfractions of *A* after $(CH_3)_2SO$ gradient: *I*, > 28 S (●——●); *II*, 18–28 S (○——○); *III*, < 20 S (▲——▲).

higher base sequence complexity than the more slowly sedimenting ones. This result would be expected if the longer and shorter molecules contained the same absolute number of globin mRNA sequences. The radioactivity distribution on the $(CH_3)_2SO$ gradients did not follow the distribution of A_{260}. While 90% of the A_{260} was reisolated from fraction III (<18 S), only 20–30% of the label appeared in this fraction (Table 2). Thus nascent or newly completed pre-mRNA chains (which became radioactive during the relatively short labeling before isolation of nuclei) behave differently from the total pool of nuclear pre-mRNA.

As a possible interpretation of the $(CH_3)_2SO$ effects, we may consider that unlabeled ("aged" or *in vivo* processed) pre-mRNA had genuine "hidden breaks" at the extremities of double-stranded "hairpin" loops (38). If these breaks represented one of the first steps of processing, it would explain why the newly-labeled (nascent or unprocessed) molecules were more stable to $(CH_3)_2SO$. It seems surprising, however, that such breaks could remain "hidden" upon treatment with hot phenol. Alternatively, it is not excluded that smaller, already processed pre-mRNA molecules formed large networks by cross-hybridization of poly(A) (28, 29) or oligo(A) (4, 27) with oligo (U) (41) segments; such a structure would break down in $(CH_3)_2SO$. We are aware that $(CH_3)_2SO$ may have effects other than simply rupturing hydrogen bonds e.g., it is a mild oxidant and may break RNA (E. Jacob, personal communication). However, it is decisive that *some* pre-mRNA molecules withstand the $(CH_3)_2SO$ treatment, sediment in $(CH_3)_2SO$ at more than 30 S, and still hybridize significantly with globin amDNA.

Conclusion

We conclude that giant pre-mRNA molecules from avian erythroblasts do indeed contain in their primary structure the nucleotide sequences of globin mRNAs isolated from this cell. This constitutes direct evidence for the hypothesis that nuclear giant, nascent messenger-like RNA molecules are in fact precursor-mRNA (3, 4, 6) and confirms our earlier experimental evidence (9), as well as recent preliminary results of others (39). However, although these data show that the globin pre-mRNA is an *informational* precursor, they do not prove that it is the direct *physical* precursor of mRNA, i.e., that the globin-specific sequence in pre-mRNA is conserved, cut out, and transported to the cytoplasm, and there serves as a template for globin synthesis. As yet no firm proof for a direct physical precursor–product relationship has been given, although labeling and chase kinetics of both pre-mRNA (4, 6, 9) and its poly(A) fraction (40) may be interpreted according to this notion. Alternative models of information processing by RNA–RNA replication, or by RNA–DNA–RNA retranscription cannot yet be ruled out.

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