Comparison of the Base-Sequence Complexities of Polysomal and Nuclear RNAs in Growing Friend Erythroleukemia Cells†

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ABSTRACT: The base-sequence complexities of polysomal poly(A)$^+$ RNA, nuclear poly(A)$^+$ RNA, and total nuclear RNA from Friend erythroleukemia cells in logarithmic phase of growth were determined by measuring the proportion of labeled unique mouse DNA sequences which formed hybrids when incubated with a vast excess of each RNA. It was estimated that the RNAs had been transcribed from 1.8, 7.6, and 8.3%, respectively, of the haploid mouse genome. Although these estimates for polysomal and nuclear poly(A)$^+$ RNAs were 2.5 times greater than those previously determined by analysis of the kinetics of the hybridization reactions between the RNAs and the cDNAs transcribed from them, they confirmed that the base-sequence complexity of nuclear poly(A)$^+$ RNA in these cells is at least four times greater than that of polysomal poly(A)$^+$ RNA.

Recent work with sea-urchin embryo nuclear and polysomal RNAs (Galau et al., 1974; Smith et al., 1974; Hough et al., 1975) demonstrated that the base-sequence complexity of the nuclear RNA is about tenfold greater than that of the polysomal RNAs, thus indicating that many of the *sequences* in HnRNA of sea-urchin embryos are confined to the nucleus. A large proportion of the poly(A)[1] synthesized in nuclei also appears to be confined to the nuclei (Perry et al., 1974), suggesting that a similar situation may apply in regard to the poly(A)$^+$ sequences in nucleus and cytoplasm. The poly(A)$^+$ RNA sequences in nuclei and on the polysomes can readily be isolated and transcribed into cDNA with reverse transcriptase; accordingly, a study of the kinetics of hybridization between poly(A)$^+$ RNAs and their cDNAs was undertaken using RNAs from a clone of Friend mouse erythroleukemia cells in logarithmic phase of growth (Birnie et al., 1974; Getz et al., 1975). These experiments indicated that posttranscriptional mechanisms alter the relative concentrations of some at least of the gene transcripts between nucleus and cytoplasm and, moreover, that there is a considerably greater number of unique DNA sequences represented in nuclear poly(A)$^+$ RNA than polysomal poly(A)$^+$ RNA. These conclusions have recently been confirmed and extended in the studies with HeLa cells (Herman et al., 1976) and *Xenopus* liver cells (Ryffel, 1976). Using the same general approach in more specific experiments, these authors demonstrated that a significant proportion of the sequences adjacent to poly(A) in nuclear RNA is not represented in cytoplasmic RNA.

One difficulty of the kinetic analyses used in our previous studies (Birnie et al., 1974; Getz et al., 1975) was that a large proportion of the RNA consisted of many copies of a relatively few sequences whereas most of the sequences were present in very low abundance. Consequently, the total base-sequence complexity of the RNAs could have been underestimated, perhaps seriously. The technique of measuring the proportion of labeled unique DNA sequences which hybridize at saturation in the presence of a large excess of RNA (Gelderman et al., 1971) is a much more sensitive method of measuring total base-sequence complexity of RNA. In view of this, we have complemented our previous work by studies of the extent to which unique mouse DNA sequences hybridize with a polysomal poly(A)$^+$, nuclear poly(A)$^+$, and total nuclear RNA from growing mouse Friend cells. Although they indicated that the base-sequence complexities of the poly(A)$^+$ RNA sequences determined previously by kinetic analysis were indeed underestimates, the data obtained confirmed our previous conclusion that the base-sequence complexity of nuclear poly(A)$^+$ RNA in these cells is at least four times greater than that of polysomal poly(A)$^+$ RNA.

Experimental Procedures

All possible precautions were taken to eliminate ribonucleases and heavy metal ions (Getz et al., 1975). All glassware used in the synthesis of cDNA and in the isolation and hybridization of RNA and DNA (including capillaries) had been siliconized (Repelcote from Hopkins and Williams Ltd., Chadwell Heath, Essex, U.K.) and then sterilized by rinsing with 0.1% aqueous diethyl pyrocarbonate and drying at 100 °C. Solutions in which hybridization reactions were done had been passed through Chelex-100 resin (Bio-Rad Laboratories, Richmond, Calif.) to remove heavy metal ions and then sterilized by treatment with diethyl pyrocarbonate; excess diethyl pyrocarbonate was destroyed by heating at 60 °C for 18 h. Suspensions of Sephadex (Pharmacia, Uppsala, Sweden) were sterilized by shaking them with diethyl pyrocarbonate (0.1%) and then heating at 60 °C for 18 h.

Cell Cultures. The origin and culture of Friend erythroleukemia cells (clone M2) used in these experiments have been described (Gilmour et al., 1974). They were grown in 4-L or 8-L stirrer cultures and were harvested in mid-log-phase of growth (0.6–0.8 × 10^6 cells/mL; mitotic index, 4–5%).

Fractionation of Cells. Nuclei were prepared from washed cell pellets by the sucrose–citric acid method (Getz et al., 1975) and stored at −20 °C. Polysomes were prepared from cells lysed with Nonidet NP-40 (Borun et al., 1967) in the presence of rat-liver ribonuclease inhibitor and purified by centrifugation through 2 M sucrose followed by rate-zonal sedimentation through 15–30% (w/w) sucrose gradients; material sedi-

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[1] Abbreviations used: poly(A), poly(adenylic acid); Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

menting at 100 S and greater was collected for preparation of polysomal RNA (Birnie et al., 1974). Chromatin was prepared from washed cell pellets as described by Gilmour et al. (1975).

Isolation of RNA. Nuclear RNA was isolated as described by Getz et al. (1975), except that each frozen pellet of nuclei from about 4×10^9 cells was thawed in 25 mL of 0.1 M Tris-HCl, pH 8, containing proteinase K (500 μg/mL) and sodium lauroyl sarcinosinate (4% w/v), suspended by homogenization and incubated at 37 °C for 45 min before exhaustive extraction with phenol–chloroform. RNA was isolated from chromatin in the same way. Polysomal RNA was prepared as described by Birnie et al. (1974). Friend-cell poly(A)$^+$ RNA was isolated from nuclear and polysomal RNA by affinity chromatography on oligo(dT)-cellulose as described by Birnie et al. (1974). Each preparation was bound twice to oligo(dT)-cellulose, recovered by precipitation with ethanol, and desalted by gel filtration through Sephadex G-25 equilibrated with sterile, distilled water. Globin mRNA was isolated from polysomal RNA from mouse reticulocytes by affinity chromatography on poly(U)-Sepharose (Harrison et al., 1974).

Transcription of Poly(A)$^+$ RNA. Polysomal poly(A)$^+$ RNA and globin mRNA were transcribed with reverse transcriptase from avian myeloblastosis virus, and the cDNA was isolated as described previously (Birnie et al., 1974). The mean size of cDNA fragments used in the experiments was 1×10^5 daltons, as determined by alkaline sucrose-gradient centrifugation (Hell et al., 1972).

Preparation of Unique Mouse [^{3}H]DNA. Highly labeled nonrepeated (unique) DNA was isolated from mouse Friend cells (clone M2) grown at 37 °C in a 250-mL spinner culture. When the cells were in mid-log-phase of growth (8×10^5 cells/mL), 2.5 mL of a mixture of aminopterin (2×10^{-5} M), deoxyadenosine (7×10^{-3} M), and glycine (3×10^{-2} M) was added, followed 15 min later by 2.5 mCi of [*methyl*-^{3}H]thymidine (40.3 Ci/mmol; Radiochemical Centre, Amersham, U.K.). The cells were collected by centrifugation 23 h later and washed with phosphate-buffered saline. DNA was isolated as described by Hell et al. (1972), sheared by ultrasonication, desalted on Sephadex G-50 (with a pad of Chelex-100) in sterile distilled water, and lyophilized. The DNA was redissolved in 0.5 M NaCl–25 mM Hepes–0.5 mM EDTA–50% formamide, pH 6.8, at 5 mg/mL, sealed in capillaries, heated at 70 °C for 5 min, and incubated at 43 °C for 4.25 h (C_0t = 250 mol s L^{-1}). Single- and double-stranded DNA were separated by hydroxylapatite column chromatography (Harrison et al., 1974); the single-stranded DNA was isolated and recycled through the hybridization and fractionation procedure. The unhybridized fraction of the DNA was finally desalted on Sephadex G-25 and lyophilized. This procedure yielded nonrepeated mouse DNA of mean single-stranded molecular weight 5×10^4 [as determined by alkaline sucrose-gradient centrifugation (Hell et al., 1972)] and specific activity 8.7×10^5 counts min^{-1} μg^{-1}.

RNA–DNA Hybridization. (i) Unique DNA. Appropriate volumes of RNA and labeled unique DNA in sterile, distilled water were mixed, lyophilized, and redissolved in 0.24 M phosphate buffer (equimolar Na_2HPO_4 and NaH_2PO_4, pH 6.8) containing 0.1% (w/v) sodium dodecyl sulfate and 1 mM EDTA. Portions of the solution were sealed in glass capillaries, denatured by heating at 100 °C for 5 min, and then incubated at 60 °C for various periods of time up to (but not exceeding) 6 days. The capillaries were flushed out with 100 volumes of 0.1 M NaCl and the solutions stored frozen at −20 °C. For analysis, each solution was divided into two equal portions. The first was fractionated by hydroxylapatite column chromatography under the same conditions used for the isolation of unique DNA, and the proportion of the radioactivity eluting as double-stranded material was determined. This material represented unique DNA in DNA–RNA hybrid plus self-annealed DNA. The second portion was incubated with ribonuclease A (50 μg/mL) at 55 °C for 2 h (conditions which have been shown to digest all the RNA in a DNA–RNA hybrid) and then fractionated by hydroxylapatite column chromatography in parallel with the first portion. The proportion of radioactivity eluting as double-stranded material from the second sample represented the self-annealed DNA alone. The difference between the proportions of radioactivity eluting as double-stranded material in the two samples is the fraction of unique DNA in RNA–DNA hybrids (Hough et al., 1975).

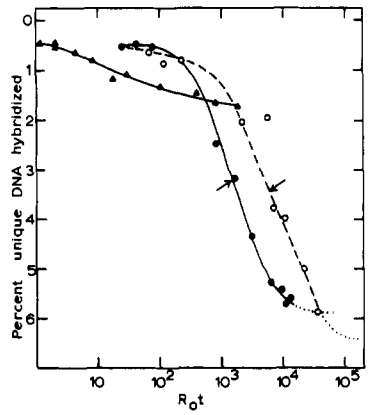

FIGURE 1: Hybridization of polysomal poly(A)$^+$ RNA (▲—▲), nuclear poly(A)$^+$ RNA (●—●), and total nuclear RNA (○- -○) with ^{3}H-labeled unique mouse DNA. Polysomal poly(A)$^+$ RNA (1.4 mg/mL), nuclear poly(A)$^+$ RNA (20 mg/mL), and total nuclear RNA (20 mg/mL) were annealed with ^{3}H-labeled unique DNA at RNA:DNA mass ratios of 1000–5000:1, and the proportion of DNA in hybrid was measured, as described in the Experimental Section. Arrows indicate $R_0t_{1/2}$ values.

(ii) cDNA. Hybridizations of globin mRNA with its cDNA were done, and the proportions of cDNA forming hybrids were measured, exactly as described for the unique DNA hybridizations. The hybridization reactions with cDNA transcribed from polysomal poly(A)$^+$ RNA were done at 43 °C in 0.5 M NaCl–25 mM Hepes–0.5 mM EDTA–50% formamide, pH 6.8, and the proportion of cDNA in hybrid was determined by measuring the proportion of radioactivity rendered acid-soluble by incubation with S_1 nuclease (Birnie et al., 1974).

Results

Estimation of Base-Sequence Complexity of RNAs. The kinetics of the hybridization reactions between ^{3}H-labeled unique mouse DNA sequences with poly(A)$^+$ polysomal RNA, poly(A)$^+$ nuclear RNA, and total nuclear RNA from mouse Friend cells are shown in Figure 1. The reaction with polysomal RNA was rapid and complete when about 2% of the unique DNA sequences had hybridized. In contrast, the reactions with the poly(A)$^+$ nuclear and total nuclear RNAs were much slower and, even at R_0t (R_0 = RNA concentration in moles of nucleotide per liter and t = time in seconds) of 10^4 mol s L^{-1} and greater, neither reaction had reached completion. Although technical limitations (solubility and stability of RNA) prevented driving these reactions further, both (in particular the one with the poly(A)$^+$ RNA) were close to completion, as is shown more clearly by the linear plots of the data in Figure 2A. Double-reciprocal plots of these data (Figure 2B) indicate that the reactions would be complete when 5.9 and 6.4% re-

TABLE I: Base-Sequence Complexities of Polysomal(A)$^+$, Nuclear Poly(A)$^+$ and Total Nuclear RNAs.

RNA	Proportion of DNA Hybridized		Proportion Unique DNA Hybridized	Base-Sequence[b] Complexity (daltons)	% of[c] Haploid Genome Transcribed
	At R_0t = 1	At Completion[a]			
Poly(A)$^+$ polysomal	0.005	0.018	0.013	1.6×10^{10}	1.8
Poly(A)$^+$ nuclear	0.005	0.059	0.054	6.8×10^{10}	7.6
Total nuclear	0.005	0.064	0.059	7.4×10^{10}	8.3

[a] From Figure 1 for polysomal RNA, Figure 2B for both nuclear RNAs. [b] Taking the molecular weight of the haploid mouse genome to be 1.8×10^{12} and assuming that 70% of the DNA consists of unique sequences. [c] Assuming transciption of DNA sequences is asymmetric.

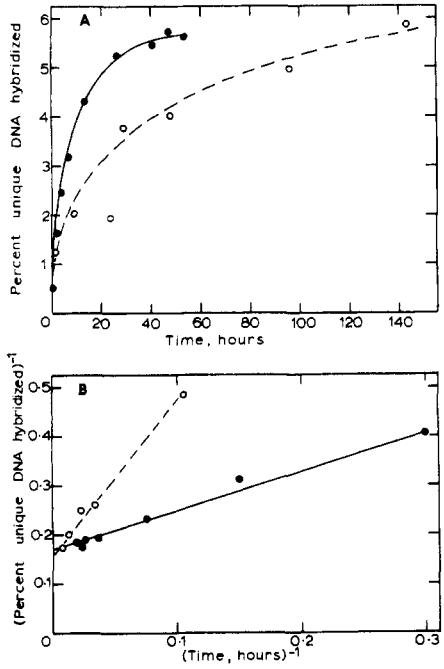

FIGURE 2: Time course of hybridization of nuclear poly(A)$^+$ RNA (●—●) and of total nuclear RNA (O- -O) with ^{3}H-labeled unique DNA. (A) Linear plot of data; (B) double-reciprocal plot of data. Hybridizations and analyses were done as described for Figure 1.

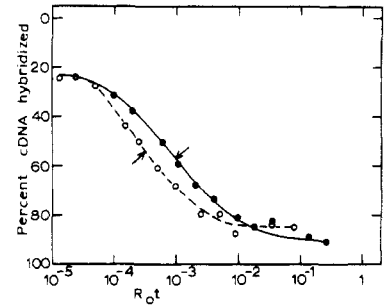

FIGURE 3: Rate of hybridization of globin cDNA with globin mRNA with (●—●) and without (O- -O) *E. coli* ribosomal RNA. Globin mRNA (0.5 μg/mL) was annealed with globin cDNA at a mass ratio of 20:1, and the proportion of cDNA in hybrid was measured, as described in Experimental Section. *E. coli* ribosomal RNA was added at 20 mg/mL (●—●); arrows indicate $R_0t_{1/2}$ values (1×10^{-3} and 3.2×10^{-4} mol s L^{-1}, respectively).

spectively [or less (Young and Paul, 1973)] of the unique DNA sequences had formed hybrids with the poly(A)$^+$ nuclear RNA and total nuclear RNA.

Figure 1 also shows that a small proportion (0.5%) of the unique DNA sequences formed hybrids with all three RNA preparations even at very low R_0t values. Hough et al. (1975) observed the same when nuclear RNA from sea-urchin embryos was hybridized to sea-urchin unique DNA and showed that the DNA in such hybrids belonged to the repetitive class of DNA sequences. Since even two cycles of partial annealing and fractionation of mouse DNA did not entirely eliminate the repetitive sequences from the preparation of unique DNA sequences (about 3% reannealed by a C_0t of 10^2 mol s L^{-1}), it is likely that the hybridization we observed at low R_0t values was also due to repetitive DNA sequences. Since RNA is probably transcribed from only a very small fraction of the repeated DNA sequences to which it can hybridize, we eliminated this from our calculations by subtracting the hybridization observed (0.5%) at very low R_0t values (1 mol s L^{-1}) from the total.

The total base-sequence complexities of the three RNA preparations, estimated from the data in Figures 1 and 2, are summarized in Table I, which shows that the sequences in polysomal poly(A)$^+$, nuclear poly(A)$^+$, and total nuclear RNA which had been transcribed from unique DNA sequences represented 1.8, 7.6, and 8.3% respectively, of the haploid mouse genome.

Estimation of the Fraction of RNA Driving the Reaction. The rate of an RNA-DNA hybridization reaction can be predicted from the base-sequence complexity of the RNA, using the reaction between globin mRNA and the cDNA transcribed from it as a kinetic standard (see Galau et al., 1974). Since the hybridization reactions with unique DNA (Figure 1) were done with RNA at very high concentrations, *E. coli* RNA at 20 mg/mL was also included in the standard globin mRNA-cDNA reaction. As shown by Figure 3, the rate of the reaction was decreased to one-third of that at low concentrations of RNA, possibly as a consequence of the greater viscosity of the reaction mixture since an increase in viscosity has been shown to decrease the rate at which DNA reanneals (Wetmur and Davidson, 1968). Aggregation of RNA might also have affected the reaction rate.

Predictions of reaction rate made in this way are only strictly correct when all the sequences in the RNA are present at equal concentrations, but they can be made without introducing significant errors when the bulk (80% or more) of the sequences in the RNA fall into a single abundance class. The shape of the curve obtained with polysomal poly(A)$^+$ RNA (Figure 1) clearly shows that the complex RNA sequences were too heterogeneous in abundances to allow it to be treated in this way. In contrast, the reactions with total and poly(A)$^+$ nuclear RNA gave curves which were reasonably close to first-order transitions, thus indicating that most of the sequences in these RNAs occupied a single abundance class of molecules.

The rate at which unique DNA should hybridize with the sequences in total nuclear RNA was calculated from their base-sequence complexity and the globin standard curve. The difference between the predicted rate and the actual rate of the

TABLE II: Analyses of Poly(A)$^+$ Nuclear and Total Nuclear RNAs.

	Poly(A)$^+$ Nuclear RNA	Total Nuclear RNA
Base-sequence complexity (daltons)[a]	6.8×10^{10}	7.4×10^{10}
$R_0t_{1/2}$(pred)[b]	170	186
$R_0t_{1/2}$(obsd)	1400	6000
Proportion of RNA driving reaction[c]	0.12	0.031
RNA per cell (g $\times 10^{-12}$)[d]	0.4	4.0
High-complexity RNA per cell (g $\times 10^{-12}$)	0.048	0.124
Molecules of RNA per sequence per cell[e]	0.43	1.0

[a] From saturation analysis (Table I). [b] Taking the base-sequence complexity of globin mRNA to be 4×10^5 daltons (Williamson et al., 1971) and the $R_0t_{1/2}$ of globin mRNA-cDNA hybridization to be 1×10^{-3} mol s L^{-1} (Figure 3). [c] Proportion of RNA driving reaction = $R_0t_{1/2}$(pred)/$R_0t_{1/2}$(obsd). [d] N. A. Affara and P. R. Harrison (personal communication) and Getz et al. (1975). [e] [High-complexity RNA/cell (g) $\times 10^{-12} \times 6 \times 10^{23}$]/[base-sequence complexity of RNA (daltons)].

TABLE III: Comparison of Kinetic and Saturation Analyses of Polysomal Poly(A)$^+$, Nuclear Poly(A)$^+$ and Total Nuclear RNAs.

	Method	Polysomal Poly(A)$^+$	Nuclear Poly(A)$^+$	Total Nuclear
% of genome represented[a]	Kinetic	0.7[b]	3.2[c]	
	Saturation	1.8[d]	7.6[d]	8.3[d]
Proportion of RNA in high-complexity class	Kinetic	0.10[b]	0.70[c]	
	Saturation		0.12 (0.24)[e]	0.031
No. of RNA molecules in high-complexity class per sequence per cell	Kinetic	3[b]	6[c]	
	Saturation		0.5 (1)[e]	1

[a] Taking the molecular weight of the haploid mouse genome to be 1.8×10^{12} and assuming 70% of the DNA consists of unique sequences. [b] From Birnie et al. (1974). [c] From Getz et al. (1975). [d] From saturation analysis (Table I). [e] Numbers in brackets recalculated assuming 50% of RNA was ribosomal sequences.

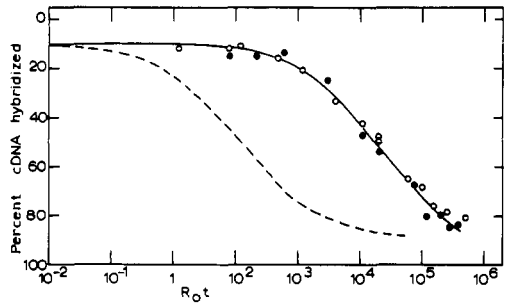

FIGURE 4: Hybridization of cDNA transcribed from Friend cell polysomal poly(A)$^+$ RNA with total nuclear RNA (○) and chromatin-associated RNA (●). RNA from nuclei and chromatin was annealed with cDNA transcribed from polysomal poly(A)$^+$ RNA at RNA:DNA mass ratios of 1000–5000:1, and the proportion of cDNA in hybrid was measured, as described in Experimental Section. The dashed line shows the hybridization of cDNA with its template polysomal poly(A)$^+$ RNA in the same conditions [data from Birnie et al. (1974)].

reaction is due to dilution of the RNA sequences driving the reaction with low-complexity RNA sequences (mainly ribosomal RNA and repetitive DNA transcripts). Hence, it was estimated (Table II) that the most complex class of sequences in nuclear RNA constituted about 3% of the mass of the RNA. Since the amount of nuclear RNA in Friend cells is known, these data also allow us to deduce that there was approximately 1 molecule of each sequence in this class per nucleus (Table II). Similar analyses of the kinetics of hybridization of unique DNA with poly(A)$^+$ nuclear RNA indicated that about 12% of this RNA contained the vast majority of the diverse sequences represented, and that there was an average of about 0.5 molecule of each sequence per nucleus (Table II).

Homology between Polysomal Poly(A)$^+$ RNA and Nuclear RNA. Although polysomal poly(A)$^+$ RNA represents a smaller proportion of the mouse genome than does nuclear RNA (Figure 1), this is not evidence that all the polysomal sequences are present in nuclear RNA. This was investigated by annealing the cDNA which had been transcribed from polysomal poly(A)$^+$ RNA with a large excess of total nuclear RNA. Figure 4 shows that the cDNA was hybridized as completely by total nuclear RNA as it was by its template polysomal poly(A)$^+$ RNA, though the rate of the reaction was about 250 times slower. This indicates that all the polysomal poly(A)$^+$ RNA sequences represented in cDNA are also present in total nuclear RNA, but at very much lower concentrations. The same results were obtained (Figure 4) when the cDNA was hybridized with RNA isolated from chromatin (endogenous chromatin RNA), indicating that this RNA contained the same concentration of polysomal poly(A)$^+$ RNA sequences as total nuclear RNA.

Discussion

The estimates of the proportions of the mouse genome represented in polyadenylated RNAs from the polysomes and nuclei of Friend cells obtained by two independent methods [namely: (i) analysis of the kinetics of hybridization between the RNAs and the cDNAs transcribed from them (Birnie et al., 1974; Getz et al., 1975); and (ii) measurement of the proportion of unique DNA hybridized by the RNAs at saturation] are compared in Table III. There is clearly a marked difference between the estimates obtained by the two methods, the saturation method giving a result which is about 2.5 times greater for both RNAs. The main reason for the discrepancy lies in the differences between the populations of RNA sequences to

which the two methods are sensitive. Measurement of the kinetics of RNA–cDNA hybridization reactions is most sensitive for analyzing the low-complexity classes of sequences present in high abundance which constitute the bulk of the RNA. It also detects high-complexity sequences present in very low abundance, but, because these RNA sequences constitute a very small proportion of the mass of the RNA, accurate estimation of the base-sequence complexity is difficult, if not impossible, by such kinetic analyses. Moreover, since these sequences constitute the most slowly hybridizing component of the mixture, measurements of the rate at which they hybridize tend to underestimate their base-sequence complexity. Consequently, the total base-sequence complexity of a population of RNA molecules determined by kinetic analysis should be regarded as a minimum estimate.

In contrast to the kinetic method, the saturation method is not sensitive to low complexity RNAs even when these sequences constitute the major portion of the mass of RNA. However, each RNA sequence is equally represented in unique DNA; consequently, measurement of the proportion of unique DNA which hybridizes with RNA is a sensitive method for estimating the base-sequence complexity of a high complexity class of molecules even though they constitute a very small proportion of the mass of the RNA. However, because of its great sensitivity, this method is particularly liable to errors arising from minor contaminations. Thus, although the polysomal RNA used in these experiments had been prepared from polysomes purified by a method known to decrease contamination by nuclear ribonucleoprotein particles to a very low level (Galau et al., 1974), it still cannot be excluded that a significant proportion of the sequences (though not of the RNA) in the polysomal RNA was derived from nuclear particles. Similarly, any contamination of the poly(A)$^+$ nuclear RNA with complex non-polyadenylated nuclear RNA sequences would cause the base-sequence complexity of this population of RNA molecules to be overestimated. Consequently, it cannot easily be excluded that these estimates of the complexity of polysomal and nuclear poly(A)$^+$ RNAs are artificially high.

In view of these limitations to both methods, precise values for the base-sequence complexities of poly(A)$^+$ polysomal and nuclear RNAs cannot yet be assigned. However, since the kinetic method tends to underestimate base-sequence complexity and the saturation method to overestimate it, it can be concluded that the polysomal poly(A)$^+$ RNA and the nuclear poly(A)$^+$ RNA of growing Friend cells are transcribed from 0.7 to 1.8% and 3.3 to 7.6%, respectively, of the haploid cell genome. Moreover, there is no significant discrepancy between the estimates of the relative base-sequence complexities of poly(A)$^+$ polysomal and nuclear RNAs, the kinetic method indicating that the base-sequence complexity of the nuclear RNA is 4.7 times that of the polysomal RNA while the saturation method indicates a 4.3-fold difference.

Poly(A)$^+$ nuclear RNA is also compared with total nuclear RNA in Table III. The results with Friend cell nuclear RNA coincide closely with those obtained by Hough et al. (1975) who concluded that the most complex class of nuclear RNA sequences in sea-urchin embryos constituted 2.5 to 3.1% of the RNA, with each of the sequences being present at about 1 molecule per cell. The data from poly(A)$^+$ nuclear RNA suggest that there is less than 1 molecule of each of the high-complexity class sequences per nucleus in Friend cells. Hough et al. (1975) also found a fractional representation of RNA sequences in sea-urchin embryos, but this can be attributed to the embryos consisting of a mixture of differentiated cells. In an exponentially growing cloned cell line, another explanation is required. One possibility is that the lifetime of some (at least) of the poly(A)$^+$ nuclear RNA sequences is less than the time between successive transcriptional events. Alternatively, the concentration of the complex class of sequences may have been underestimated, either because they are not completely homogeneous in abundance or because the poly(A)$^+$ nuclear RNAs are contaminated with low complexity nonpolyadenylated RNA sequences, specifically ribosomal RNA. It has been found that affinity chromatography on oligo(dT)-cellulose may be considerably less effective than poly(U)-Sepharose [which was used previously by Getz et al. (1975)] at reducing the concentration of ribosomal RNA sequences to negligible levels. Frequently 10% and, on occasion, up to 50% of an RNA which had been bound twice to oligo(dT)-cellulose has been found to be ribosomal RNA (A. J. Minty, personal communication). Contamination of the poly(A)$^+$ RNA with ribosomal RNA will have no effect on the estimate of base-sequence complexity by saturation analysis, but will cause the observed rate of the reaction with the complex class of poly(A)$^+$ sequences to be artificially low, resulting in the proportion of poly(A)$^+$ RNA sequences driving the reaction to be underestimated.

The estimate of the difference between poly(A)$^+$ and total nuclear RNA (Table III) must be taken as a minimum one because of the possibility of contamination of the poly(A)$^+$ RNA with nonpolyadenylated sequences. That there should be a difference between these RNAs, both in base-sequence complexity and in the proportion consisting of the most complex class of sequences, is not unexpected. Similar and larger differences have recently been reported for mouse brain RNAs (Bantle and Hahn, 1976).

The data in these experiments, taken together with those obtained previously by analysis of the kinetics of hybridization between poly(A)$^+$ RNAs and their cDNAs (Birnie et al., 1974; Getz et al., 1975), give a partial picture of the steady-state composition of polysomal poly(a)$^+$, nuclear poly(A)$^+$, and total nuclear RNA in growing Friend cells. Comparison of the data obtained by the two methods clearly indicates that, when both are used together, considerably more information about an RNA population can be obtained than from either alone. It is now clear that the sequences in poly(A)$^+$ nuclear RNA are less complex than those in nuclear RNA, while those in poly(A)$^+$ polysomal RNA, although all represented in nuclear RNA, are only 20–25% as complex as those in poly(A)$^+$ nuclear RNA. The confirmation by saturation analysis of our previous conclusion (Getz et al., 1975) that the nuclear poly(A)$^+$ RNA sequences are more complex than polysomal poly(A)$^+$ RNA sequences is in agreement with similar data obtained with RNA from sea-urchin embryos (Galau et al., 1974; Hough et al., 1975) and mouse brain (Bantle and Hahn, 1976). These data, together with the recent demonstration that a significant proportion of the sequences adjacent to poly(A) in nuclear RNA are not represented in cytoplasmic RNA either in HeLa cells (Herman et al., 1976) or in *Xenopus* liver (Ryffel, 1976), provide more evidence in favor of the suggestion that there is qualitative (Scherrer and Marcaud, 1968; Darnell et al., 1973; Scherrer, 1974; Getz et al., 1975) as well as quantitative (Ryffel and McCarthy, 1975; Humphries et al., 1976; Young et al., 1976) control of gene expression at a posttranscriptional level in eukaryotic cells.

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Characterization of Dog Small Intestinal Fucolipids with Human Blood Group A Activity. Differences in Dog and Human A-Active Fucolipids†

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ABSTRACT: Glycolipids containing fucose (fucolipids) which carried human blood group A activity were isolated from a number of dog small intestines and analyzed. On the basis of sugar analysis, methylation, periodate oxidation, enzyme degradation, mass spectrometry, and immunologic studies, a structure is proposed for these substances. The ceramides of the dog fucolipids contained only hydroxylated fatty acids with 85% saturated and 15% monoenoic acids ranging from 16 to 25 carbon atoms. Sphingosine and phytosphingosine comprised 48% each of the long chain bases. An A-active fraction isolated from human small intestine was shown to have two components, one of which was immunologically distinct and the other identical with the dog intestinal fucolipids. The human fraction differed from the dog fucolipids in migration on thin-layer chromatography and contained two types of amino sugar substitution. It is proposed that the human fraction was composed of two fucolipids with incomplete structures.

The possibility that the A antigen of the human ABO (H) blood group system exists on erythrocytes as a number of variants has long been recognized (Race and Sanger, 1962; Hakomori and Strycharz, 1968; Koscielak et al., 1970; Hakomori et al., 1972). The human A antigens are also distributed on erythrocytes and/or other tissues of infrahuman species, although there is some evidence for antigenic differences in these antigens among the species (Sorensen et al., 1974; Joysey, 1959; Furuhata, 1962; Borel, 1954; Schroffel et al., 1971; Slomiany and Horowitz, 1972, 1973; Thiele and Koch, 1973; Hiramoto et al., 1973; Zweibaum et al., 1974b; Slomiany et al., 1973, 1974; Slomiany and Slomiany, 1975; Hakomori and Watanabe, 1976; Slomiany et al., 1976a, 1976b). The variations in A antigenicity which are due to structural differences and those which are due to organizational differences on the

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