# Comparison of Complexity and Diversity of Polyadenylated Polysomal and Informosomal Messenger Ribonucleic Acid from Chinese Hamster Cells†

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ABSTRACT: The sequence complexity and relative abundance of cytoplasmic polyadenylated polysomal (ribosome-bound) mRNA and cytoplasmic polyadenylated informosomal (ribosome-free) mRNA were analyzed in exponentially growing Chinese hamster cells (line CHO) using the technique of cDNA hybridization to excess poly(A)$^+$ mRNA. Polysomal and informosomal mRNAs had similar complexities (~8300 mRNA species), but both the fraction of mRNA and the number of sequences comprising the mRNA abundance classes were different. Heterologous annealing reactions showed that all of the mRNA sequences detected were shared by the polysomal and informosomal mRNAs. However, the most abundant informosomal mRNA component was considerably different from the most abundant polysomal mRNA component. For a more detailed analysis, cDNA complementary to the most abundant informosomal and polysomal mRNAs was isolated. By use of the fractionated cDNA, it could be demonstrated that the most abundant informosomal mRNA sequences were distributed in the polysomal mRNA with an approximately fivefold reduction in relative frequency. These results are not compatible with models postulating translational control of gene expression by the complete sequestering of some mRNA sequences in an untranslatable form in the cytoplasm. The data are, however, consistent with models encompassing differential rates of initiation on the polysome and/or preferential affinity of some mRNAs for initiation factors.

In eucaryotic cells, a fraction of the cytoplasmic mRNA[1] is associated with protein but is not associated with ribosomes. It has been postulated that these ribosome-free ribonucleoproteins (informosomes) represent a storage form of mRNA in the cytoplasm until such time that ribosome association and subsequent translation of the message are required (Henshaw, 1968; Spirin, 1969; Spohr et al., 1970). Thus, the informosomes have the potential for exerting control of gene expression at the translational level. A number of studies in developing and differentiating systems support such a role for informosomes: preexisting or newly synthesized messenger ribonucleoprotein (mRNP) can remain in the cytoplasm for some time prior to ribosome association and translation (Terman, 1970; Felicetti et al., 1975; Buckingham et al., 1976; Iatrou & Dixon, 1977; Slegers & Kondo, 1977; Iatrou et al., 1978); defined messengers, though present both in ribosome-free and ribosome-bound states in the cytoplasm (Gander et al., 1973; Bag & Sarker, 1976; Zähringer et al., 1976; Heywood & Kennedy, 1976), may be partitioned differently from that of the total mRNA population (Buckingham et al., 1976; Zähringer et al., 1976); and under the appropriate stimulus, the distribution of some mRNA sequences between the ribosome-free and -bound states can be altered (Bandman & Gurney, 1975; Rudland et al., 1975; Yap et al., 1978).

While the results with developing and differentiating systems provide the basis for some hypotheses, the fact remains that rapidly dividing cells in culture may also have from one-third to one-half of their cytoplasmic mRNP unassociated with ribosomes (Spohr et al., 1970; Enger & Hanners, 1978). The question arises as to what role the informosomes play in such cells whose capacity to perform differentiated functions is limited if not altogether absent. In particular, it would be useful to know the complexity and degree of diversity of the polysomal and informosomal cytoplasmic mRNA populations in evaluating the role that informosomes may play in translational control. Although this problem has been addressed to a limited extent, divergent results have been obtained. Levy W & Rizzino (1977) reported that a significant portion of the total cytoplasmic polyadenylated RNA sequences was not bound to polysomes of *Drosophilia* tissue culture cells. However, Goldstein (1978) found that the ribosome-free and ribosome-bound mRNA population from *Drosophila* oocytes and embryos contained substantially the same sequences. In both of the above studies, the conclusions were based on comparisons of annealing of cDNA copies of total cytoplasmic polyadenylated mRNA (polysomal + informosomal mRNA) with either polysomal or informosomal mRNA (e.g., no comparisons were made with cDNA copies of purified polysomal or informosomal mRNA). This protocol has limitations in that (1) informosomal mRNA can constitute a variable portion of the total cytoplasmic mRNA in different cells and (2) no information can be obtained on either the complexity of the polysomal and informosomal mRNA fractions or the frequency (and possible changes therein) at which classes of messengers are represented in the different mRNA populations. We report here the results of our work obtained from annealing cDNA copies of polyadenylated polysomal and informosomal mRNA to a vast excess of polyadenylated mRNA from Chinese hamster cells (line CHO). We found that (1) polysomal and informosomal mRNA populations had similar complexities but the relative frequency distribution was different for the two populations, (2) all of the different mRNA sequences detected were present in both the polysomal and informosomal mRNA fractions, and (3) the most abundant informosomal mRNAs were found in the polysomal mRNA at a considerably lower relative frequency. Our results are not compatible with models postulating translational control by the complete or nearly complete

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[1] Abbreviations used: poly(A)$^+$, polyadenylated; buffers, K = KCl, T = Tris, N = NaCl, M = $MgCl_2$, S = sodium dodecyl sulfate ($NaDodSO_4$), and E = ethylenediaminetetraacetic acid (EDTA) (the subscript numbers indicate millimolar concentrations or percent $NaDodSO_4$); $Cl_3AcOH$, trichloroacetic acid; cDNA, complementary DNA; mRNP, messenger ribonucleoprotein; mRNA, messenger ribonucleic acid.

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sequestering of many mRNA sequences in an untranslatable form in the cytoplasm.

## Experimental Procedures

*Cell Culture.* Chinese hamster cells (line CHO) were cultured as previously described (Walters & Ratliff, 1975). The cultures were negative for *Mycoplasma* contamination (House & Waddell, 1967). Cell doubling times ranged from 16.5 to 18.0 h. Cells were seeded at $\sim 1 \times 10^5$ cells/mL and were harvested at $(4\text{–}4.25) \times 10^5$ cells/mL while still in exponential growth.

*Preparation of Poly(A)$^+$ Polysomal and Informosomal mRNA.* Cells were harvested by pouring culture aliquots over frozen 0.25 M sucrose. The chilled cells were washed by sedimentation through cold 0.25 M sucrose. The cell pellet was suspended in $K_{10}T_{10}M_{1.5}$ buffer (10 mM KCl, 10 mM Tris, pH 7.5 at 25 °C, and 1.5 mM $MgCl_2$). Cells from 320 mL of culture were frozen in 1.6 mL of buffer. Frozen cell suspensions were stored at −70 °C.

To prepare cytoplasm, we thawed and brought frozen cell suspensions to ice-bath temperatures. Nonidet P-40 nonionic detergent (0.2 mL, 10%, Shell Oil Co.) was added, and the tube was agitated for 10 s by using a vortex agitator and placed in ice for 15 min. After another 10 s of agitation, 0.2 mL of 5% sodium deoxycholate was added; the suspension of lysed cells was again agitated for 10 s, allowed to stand in ice for 20 min, and agitated for 20 s. The nuclei were then removed by sedimentation (25 min at 2000 rpm in a CRU-5000 IEC centrifuge). The supernatant (cytoplasm) was drawn off and used to prepare RNA as described below.

To prepare total (informosomal plus polysomal) cytoplasmic mRNA, we placed the cytoplasm in an A211 IEC centrifuge tube, underlaid with 1.5 mL of 1 M sucrose in $K_{10}T_{10}M_{1.5}$, and polysomes and informosomes were pelleted by centrifugation for 3 h at 140000*g* average. The resulting pellet was rinsed 5 times with distilled water and then suspended in 10 mL of $N_{100}T_{10}S_{0.5}E_{10}$ and extracted once with chloroform–phenol (1:1) and twice with chloroform–isoamyl alcohol (24:1) followed by addition of 2.5 volumes of 95% ethanol to precipitate the RNA. Poly(A)-containing mRNA was then isolated as described below.

The cytoplasm, prepared as above, was treated with 0.3 mL of $K_{800}T_{10}M_{1.5}$, mixed, and placed on ice to resolve ribosomes and polysomes; 0.25 mL of 10% sodium deoxycholate was added, and the mixture was held on ice another 5 min. The cytoplasm was then layered on a 10–50% exponential sucrose gradient ($K_{100}T_{10}M_{1.5}$ buffer, volume 56 mL, Beckman Spinco SW25.2 rotor tube) and centrifuged for 3.5 h at 24 000 rpm at 4 °C. After centrifugation, the gradient was fractionated by pumping through a monitor to record absorbancy and fractions containing informosomal (Figure 2, region R) and polysomal (Figure 2, region P) mRNA were taken. Isopycnic centrifugation in cesium chloride of formaldehyde-fixed aliquots from sucrose gradients was performed as previously described (Enger et al., 1973).

To prepare polysomal and informosomal RNA, we mixed sucrose gradient fractions from region R and region P (Figure 2) with bentonite (0.15 mL of 250 mg/mL per 15–16 mL of gradient fraction) and then precipitated them with 2.5 volumes of ethanol. The ethanol precipitate (and bentonite) was spun down, suspended in 10 mL of $N_{100}T_{10}E_{10}S_{0.5}$ (100 mM NaCl, 10 mM Tris, pH 7.4 at 25 °C, 10 mM EDTA, and 0.5% sodium dodecyl sulfate), and extracted with an equal volume of chloroform–phenol (1:1). The aqueous fraction was then extracted twice with chloroform–isoamyl alcohol (24:1) and precipitated with 2.5 volumes of ethanol. RNAs were reprecipitated after solution in $N_{100}T_{10}E_1S_{0.1}$. RNAs from 18 sucrose gradients usually were pooled at the RNA extraction and precipitation stages.

To prepare poly(A)$^+$ mRNA, we dissolved extracted and reprecipitated RNAs in $N_{120}T_{10}E_1S_{0.5}$ buffer and applied the solution to a column of oligo(dT)–cellulose. The 2 × 10 cm column contained 4 g of oligo(dT)–cellulose (Collaborative Research, T3 grade) that was washed with 0.1 M NaOH and then with water and 0.5% $NaDodSO_4$ and equilibrated with $N_{120}T_{10}E_1S_{0.05}$ (120 mM NaCl, 10 mM Tris, pH 7.5 at 25 °C, 1 mM EDTA, and 0.05% $NaDodSO_4$). Poly(A)$^-$ RNA was eluted to 40 mL with starting buffer. Poly(A)$^+$ RNA was then eluted with 0.05% $NaDodSO_4$. This eluate was heated for 2 min at 60 °C, adjusted to $N_{120}T_{10}E_1S_{0.5}$, applied again to oligo(dT)–cellulose, washed with $N_{120}T_{10}E_1S_{0.05}$, eluted with 0.05% $NaDodSO_4$, extracted with chloroform–phenol and then with chloroform–isoamyl alcohol to remove $NaDodSO_4$, and then precipitated with 2.5 volumes of ethanol. After 3 days, the precipitate was collected by centrifugation. The pellet was taken up in sterile distilled water and the concentration measured by ultraviolet absorbance at 260 nm (1 OD/mL was taken as 40 μg/mL of RNA). From earlier work, the size of informosomal and polysomal poly(A)$^+$ RNA was estimated to be 1500–2000 nucleotides (M. D. Enger and R. A. Walters, unpublished experiments). This RNA was used as a template for synthesis of complementary DNA (cDNA) and as a driver in annealing reactions with cDNA. Hereafter, any references to mRNA used in the experiments described below will be taken to mean polyadenylated mRNA. No experiments were performed with nonpolyadenylated mRNA.

*Synthesis of cDNA.* The reaction mixtures, 0.15 mL in volume, contained the following components: 50 mM Trizma (pH 8.5, Sigma); 20 mM dithiothreitol; 6 mM $MgCl_2$; 100 mM NaCl; 240 μM each dATP, dGTP, and dTTP; 50 μM [$^3$H]dCTP (24 Ci/mmol, New England Nuclear); 50 μg/mL actinomycin D; 5 μg/mL oligo(dT)$_{12\text{–}18}$ (Miles); 213 units/mL of avian myeloblastosis virus RNA-dependent DNA polymerase (provided by Dr. J. Beard, Life Sciences, Inc., St. Petersburg, FL); and 20 μg/mL poly(A)$^+$ mRNA. The reaction mixture was incubated at 37 °C for 30 min. If the cDNA was to be used in annealing experiments, the reaction was terminated by heating to 100 °C for 2 min followed by quick cooling on ice. The RNA was hydrolyzed with 0.2 N NaOH at 75 °C for 15 min, and the cDNA was recovered as described by Weiss et al. (1976). The yield of cDNA ranged from 22 to 28% of the input mRNA based on the specific radioactivity of the [$^3$H]dCTP and assuming equal representation of all four bases. The specific radioactivity of the cDNA was $\sim 1.5 \times 10^7$ cpm/μg.

If the size of the cDNA was to be estimated, the synthesis reaction was terminated by adding 20-μL aliquots of the reaction mixture to an ice-cold 1.0-mL solution with a final concentration of 1.0 M KCl and 50 μg of yeast tRNA. The samples were precipitated with 95% ethanol overnight. The precipitate was collected by centrifugation, taken up in 0.25 mL of 0.1 M KCl–10 mM EDTA (pH 7.0), and dialyzed at 4 °C against the same solution (24 h, two changes, each 1 L). The cDNA at this stage was 85–90% resistant to $S_1$ nuclease digestion. Samples (20 μL) were taken for alkaline sucrose density gradient centrifugation under conditions described previously (Walters & Hildebrand, 1975). The number average molecular weight of the cDNA was estimated from 24 gradients of cDNA copied from eight different polysomal, informosomal, and total cytoplasmic poly(A)$^+$ mRNA preparations. The estimated size of cDNA preparations

ranged from 1300 to 1800 nucleotides in length. The variation in estimated nucleotide length was as great between cDNAs copied from the same mRNA fraction as that from different mRNA fractions. Thus, we could detect no size differences among cDNAs copied from polysomal, informosomal, or total cytoplasmic poly(A)$^+$ mRNA preparations.

*cDNA–mRNA Hybridization.* Hybridization of cDNA with excess poly(A)$^+$ mRNA was performed essentially as described by Bishop et al. (1974a), although some modifications were made. All solutions and glassware were sterilized prior to use. The hybridization mixture contained the following components: 10 mM Trizma (pH 7.7, Sigma), 2 mM EDTA, 360 mM NaCl, 0.1% (w/v) $NaDodSO_4$, 0.5 $\mu$g/$\mu$L yeast tRNA (Miles), 1000 cpm cDNA/assay, and varying amounts of mRNA. Hybridizations were performed with 5-$\mu$L samples overlaid with mineral oil in small glass conical tubes that had been treated with dichlorodimethylsilane (Aldrich) and 30% hydrogen peroxide prior to use. After being heated at 100 °C for 3 min, samples were incubated at 68 °C to reach the desired $R_0t$ value [product of initial RNA concentration in moles of nucleotide per liter (330 g/mol nucleotide) and time in seconds]. Reactions were terminated by addition of 0.5 mL of $S_1$ nuclease buffer (pH 4.5, 30 mM sodium acetate, 3 mM $ZnCl_2$, 300 mM NaCl, 10 $\mu$g/mL denatured calf thymus DNA, and 10 $\mu$g/mL native calf thymus DNA). The amount of cDNA–mRNA hybrid was immediately determined by resistance to *Aspergillus oryzae* $S_1$ nuclease (Miles). Duplicate 100-$\mu$L aliquots were removed from the sample and spotted onto GF/C filters (Whatman). $S_1$ nuclease (278 units) was added to the remainder of the sample, and the sample was incubated for 60 min at 37 °C. Following incubation, duplicate 100-$\mu$L aliquots were removed and spotted on filters. Immediately following sample addition, the filters were immersed in ice-cold 10% $Cl_3AcOH$–0.01 M sodium pyrophosphate and then individually washed under suction 6 times with ice-cold absolute ethanol. The filters were air-dried, and the radioactivity was determined by liquid scintillation counting as previously described (Walters et al., 1974). The fraction of hybridized cDNA was determined by comparing the acid-precipitable radioactivity in the undigested samples with that of the $S_1$ nuclease digested samples.

Zero time control samples were taken, and the fraction of cDNA remaining after $S_1$ nuclease digestion was subtracted from the experimental data points. We routinely found that 2–3% of the cDNA was $S_1$ nuclease resistant.

All values of $R_0t$ are those actually observed under our reaction conditions (360 mM $Na^+$). No corrections to standard conditions (180 mM $Na^+$) have been made. If one wishes to do so, consult the salt correction factors given by Britten et al. (1974).

*Poly(A) Titration with Poly(U).* Because we wished to compare the results of hybridization of different fractions of the cytoplasmic mRNA, we thought it necessary to demonstrate that the fractions were of equivalent purity. To this end, the poly(A) content of our RNA preparations was determined by titration with radioactive poly(U) as described by Bishop et al. (1974b). A standard curve was constructed by titrating known amounts of $poly(A)_{130}$ (Miles) with [$^3$H]poly(U) (6.74 Ci/mmol, New England Nuclear). By comparing the amount of [$^3$H]poly(U) bound by our RNA preparations with the standard curve, we determined that 5.7 ± 0.3% of the mRNA preparations (by weight) was contributed by poly(A). This value is within the range reported by others (Hastie & Bishop, 1976; Sala-Trepat et al., 1978) and suggests that our mRNA preparations were reasonably pure. More importantly, however, we found no difference in the poly(A) content of our polysomal, informosomal, or total cytoplasmic mRNA samples, which indicates that, whatever the absolute purity, the different RNA preparations were essentially equivalent.

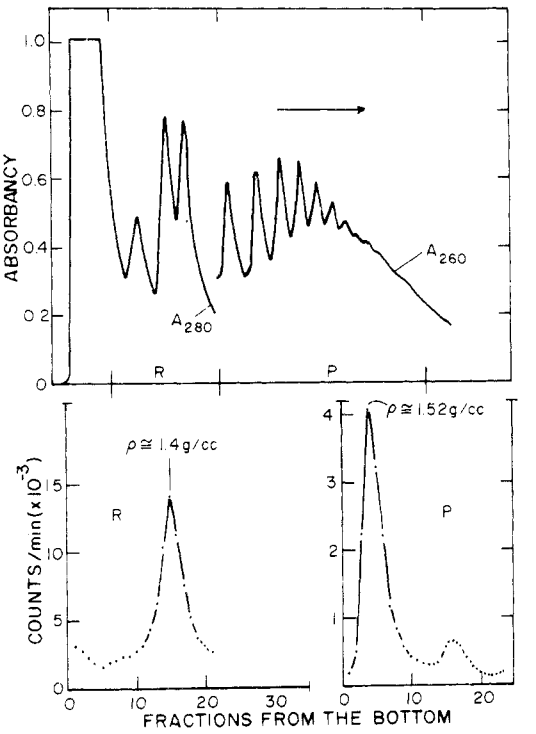


FIGURE 1: (Top) Absorbancy pattern of cytoplasm prepared by using 1% NP-40 and 0.5% sodium deoxycholate resolved into ribosome (R) and polysome (P) regions by sedimentation through a 10–50% exponential sucrose gradient. Cells were exposed to 1.5 $\mu$Ci/mL [5-$^3$H]uridine (Schwarz/Mann, 4 Ci/mmol) for 6 h after 30-min pretreatment with 0.05 $\mu$g/mL actinomycin D. (Bottom) Counts per minute distribution of formaldehyde-fixed aliquots of R or P regions of sucrose gradient after isopycnic centrifugation in cesium chloride. Peak at $\rho \simeq 1.4$ g/cm$^3$ represents labeled mRNA in informosomes and that at $\rho \simeq 1.52$ g/cm$^3$ represents labeled mRNA in polysomes.

## Results

*Resolution of Informosomes and Polysomes.* An estimate of the efficiency of resolution of informosomal and polysomal fractions was obtained from cells labeled with [$^3$H]uridine for 6 h in the presence of 0.05 $\mu$g/mL actinomycin D. This protocol was chosen because (1) actinomycin D preferentially inhibits incorporation into rRNA but allows incorporation into mRNA (Enger & Campbell, 1975) and (2) short pulse-label times overestimate informosome contamination of polysomes as label enters informosomes 15 min before it appears in polysomes (Enger & Hanners, 1978). Zone sedimentation of cytoplasm prepared by using 1% NP-40 and 0.5% sodium deoxycholate resolved ribosomes and ribosomal subunits (Figure 1, region R) from polysomes (Figure 1, region P). Formaldehyde-fixed aliquots of the R and P regions from the sucrose gradient were analyzed by isopycnic centrifugation in cesium chloride for the type of mRNP present in each region. It can be seen that the only mRNA present in the R region was in informosomes (Figure 1R). However, the P region contained mRNA from both polysomes and informosomes (Figure 1P). We estimated that ~18% of the mRNA of the P region was informosomal mRNA contamination. Therefore, the problem was to reduce or eliminate this contamination of polysomal mRNP with informosomes. This was achieved by adjusting the deoxycholate concentration of the cytoplasm to 1.3% prior to sucrose gradient centrifugation, an example of which is shown in Figure 2. Under these conditions, little or

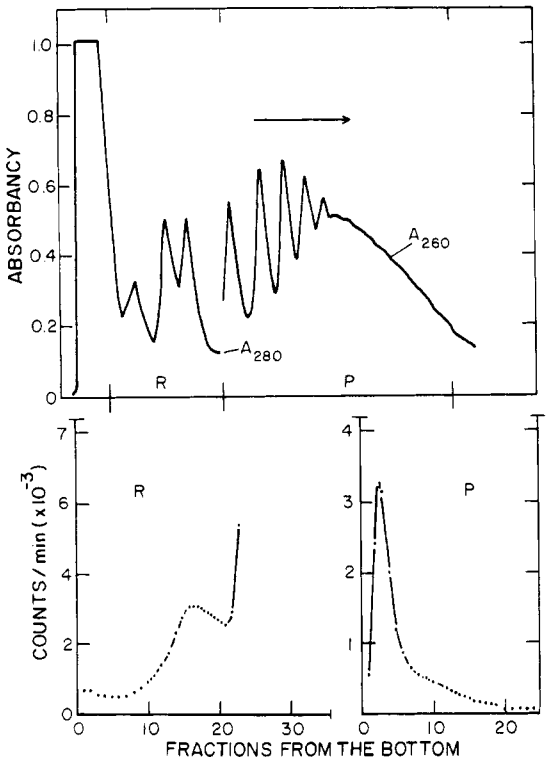


FIGURE 2: (Top) Absorbancy pattern of cytoplasm prepared as in Figure 1 but where the concentration of sodium deoxycholate was increased to 1.3% 5 min before layering onto sucrose gradient. Cells were exposed to 2 $\mu$Ci/mL [2,8-$^3H_2$]adenosine (New England Nuclear, 5 mCi/0.043 mg) for 90 min after 30-min pretreatment with 0.05 $\mu$g/mL actinomycin D. (Bottom) Counts per minute distribution of formaldehyde-fixed aliquots of R or P regions of sucrose gradient after isopycnic centrifugation. Centrifugation conditions were the same as those in Figure 1.

no detectable contamination of polysomes with informosomes was evident, even after a short (90 min) labeling period (compare Figure 2P with Figure 1P). Of the total poly(A)$^+$ mRNA isolated from cytoplasm prepared as above, ~68% was ribosome bound (polysomal) and ~32% was ribosome free (informosomal).

*Hybridization of Total Cytoplasmic cDNA with Informosomal and Polysomal mRNA.* cDNA copied from total cytoplasmic poly(A)$^+$ mRNA (polysomal + informosomal mRNA) was annealed with excess total cytoplasmic mRNA (Figure 3A), polysomal mRNA (Figure 3B), and informosomal mRNA (Figure 3C). Inspection of parts A–C of Figure 3 shows little or no difference in the amount of cDNA hybridized at saturation (~85%). When the data from parts A–C of Figure 3 are compared as shown in Figure 3D, it is apparent that cDNA hybridization kinetics of reactions driven with either total cytoplasmic or polysomal mRNAs were virtually indistinguishable, reflecting the preponderance of polysomal mRNA in total cytoplasmic mRNA. The kinetics of the reaction driven by informosomal mRNA did, however, appear to be somewhat different. The equivalent saturation values suggest that all (hybridizing) sequences contained in the polysomal mRNA population are also present in the informosomal mRNA population. The differences in the shapes of the curves for informosomal and polysomal mRNA hybridized with cDNA to total cytoplasmic mRNA suggest a difference in relative abundance of common species within each population. This is better illustrated by using cDNAs to the individual populations.

*Hybridization of Polysomal and Informosomal cDNAs with Their Respective Templates.* Figure 4A shows the hybridization of chicken globin and polysomal cDNAs with their respective mRNAs, and Figure 4B shows the hybridization of informosomal cDNA with its mRNA. As expected, the hybridization of polysomal and informosomal cDNAs to their respective templates was much slower and the curves were much broader when compared to the chicken globin standards. In addition, the homologous informosomal and polysomal annealing reactions are substantially different from each other. A portion of the informosomal cDNA anneals considerably faster than polysomal cDNA (Figure 4B).

An estimation of the number of abundance classes in each population (parts A and B of Figure 4) was made by the use of a series of linear plots of percent cDNA hybridized vs. $R_0t$,

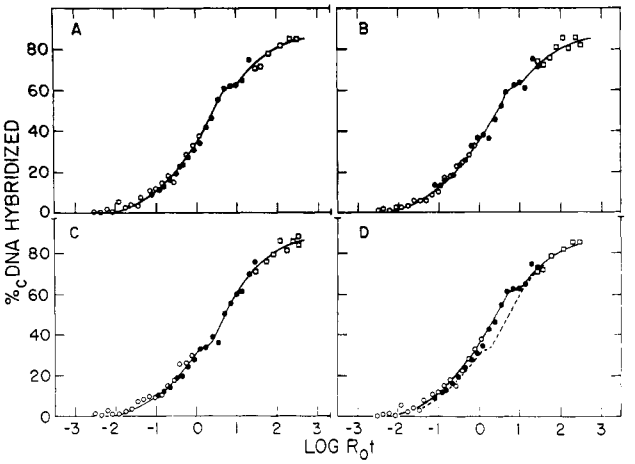


FIGURE 3: Hybridization of total cytoplasmic cDNA with (A) total cytoplasmic poly(A)$^+$ mRNA, (B) polysomal poly(A)$^+$ mRNA, and (C) informosomal poly(A)$^+$ mRNA [(○) 18 $\mu$g/mL RNA, (●) 36 $\mu$g/mL RNA, (□) 300 $\mu$g/mL RNA]. (D) Comparison of reaction kinetics from the data shown in panels A–C. The data points are taken from panel A, the solid curve is the polysomal mRNA driven reaction reproduced from panel B, and the dashed curve is the informosomal mRNA driven reaction reproduced from panel C.

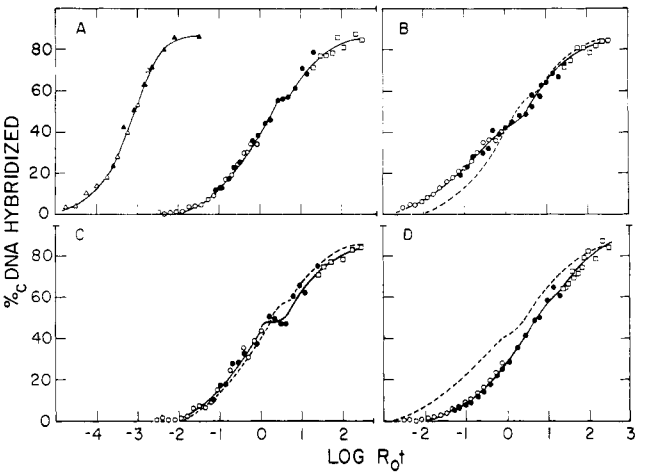


FIGURE 4: Hybridization of polysomal and informosomal cDNAs. (A) Homologous hybridizations of the chicken globin standard poly(A)$^+$ mRNA [(△) 0.326 $\mu$g/mL RNA, (▲) 3.26 $\mu$g/mL RNA] and polysomal poly(A)$^+$ mRNA [(○) 18 $\mu$g/mL RNA, (●) 36 $\mu$g/mL RNA, (□) 300 $\mu$g/mL RNA] with their respective cDNAs. (B) Homologous hybridization of informosomal poly(A)$^+$ mRNA with its cDNA; (---) homologous polysomal curve from panel A is given for comparison. (C) Heterologous hybridization of polysomal poly(A)$^+$ mRNA with informosomal cDNA; (---) homologous polysomal curve from panel A is given for comparison. (D) Heterologous hybridization of informosomal poly(A)$^+$ mRNA with polysomal cDNA; (---) homologous informosomal curve from panel B is given for comparison. Data points in panels B–D are (○) 18 $\mu$g/mL RNA, (●) 36 $\mu$g/mL RNA, and (□) 300 $\mu$g/mL RNA.

Table I: Poly(A)$^+$ mRNA Complexity Calculated from Hybridization Data[a]

| source of RNA | class | fraction of hybridizable cDNA | obsd $R_0t_{1/2}$ | cor $R_0t_{1/2}$ | no. of different mRNA sequences[b] |
|---|---|---|---|---|---|
| polysomes | abundant | 0.665 | 0.347 | 0.231 | 385 |
| | scarce | 0.335 | 14.13 | 4.73 | 7883 |
| | | | | | 8268[c] |
| informosomes | abundant | 0.529 | 0.085 | 0.045 | 75 |
| | scarce | 0.471 | 10.47 | 4.93 | 8217 |
| | | | | | 8292[c] |

[a] Data from parts A and B of Figure 4 were analyzed as described by Bishop et al. (1974a) using chicken globin mRNA as a standard. [b] No corrections have been made for the effect of cDNA size on the rate of hybridization. Assuming that our cDNA was 1300–1800 nucleotides long and that the chicken globin cDNA was full copy, our estimate of the number of sequences could be low by 40–70% if rate is proportional to the square root of the length of cDNA (Wetmur & Davidson, 1968). In practice, however, we are reluctant to make such a correction until such time that kinetic measurements have been made with cDNAs of a number of well-defined lengths copied from a single mRNA species. [c] Total.

and the sequence complexity was calculated as described by Bishop et al. (1974a). Under our hybridization conditions, the $R_0t_{1/2}$ of the chicken globin reaction was $6.0 \times 10^{-4}$ mol $L^{-1}$ s. The complexity of chicken globin mRNA was assumed to be 1820 nucleotides (Harding et al., 1977). The results of our analysis are presented in Table I. Only two abundance groups could be detected in the polysomal and informosomal mRNA. Although the relative mRNA abundance is different, informosomal and polysomal mRNA have nearly the same calculated sequence complexity.

*Heterologous Annealing Reactions.* Figure 4C shows the hybridization of informosomal cDNA with polysomal mRNA. It can be seen that polysomal mRNA could drive essentially all of the informosomal cDNA into hybrid form with kinetics that are very similar to those of the polysomal mRNA/cDNA homologous reaction. Since approximately the same fraction of informosomal cDNA is hybridized at low $R_0t$ with polysomal as with informosomal mRNA (e.g., compare the inflection points in parts B and C of Figure 4), one suspects that those sequences which are most abundant in informosomal mRNA (and its cDNA) are present in the abundant fraction of polysomal mRNA. If in addition the data presented in Table I are correct in estimating the number of sequences present in the abundant polysomal and informosomal mRNA fractions, then the most abundant informosomal mRNA sequences would be represented in the polysomal mRNA at about one-fifth the relative frequency (75:385, Table I).

Figure 4D shows the hybridization of polysomal cDNA with informosomal mRNA. Again, informosomal mRNA could drive all of the polysomal cDNA into hybrid form, but the reaction kinetics were quite different from those of the informosomal mRNA/cDNA homologous reaction. It will be noted that hybridization of the polysomal cDNA to informosomal mRNA was reduced primarily in the abundant informosomal mRNA component. This suggests that the abundant informosomal mRNA is different from the abundant polysomal mRNA. This kind of analysis is reminiscent of that reported by Hastie & Bishop (1976) demonstrating differences in the composition of the abundant components of mRNA from mouse liver, kidney, and brain tissues.

The data in parts C and D of Figure 4 show that, within the limits of the technique, all of the sequences detected were present in both polysomal and informosomal mRNA. That is, cDNA to polysomal mRNA reacts to the same extent with either polysomal or informosomal mRNA and cDNA to informosomal mRNA is similarly saturated with polysomal and informosomal mRNAs. This being the case, the kinetics of the heterologous reactions must be a consequence of differences in the relative abundance of some sequences between

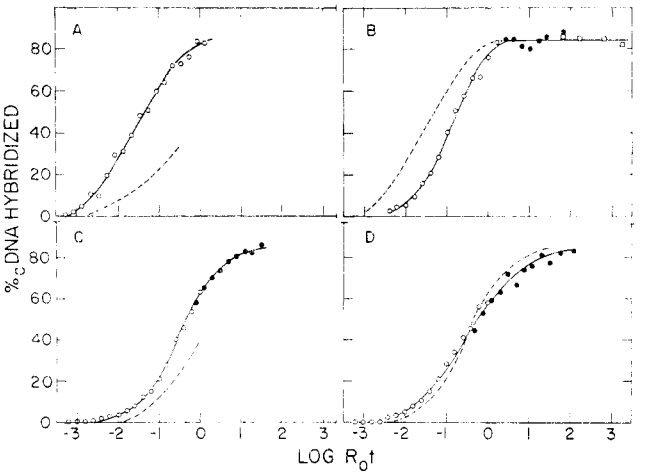


FIGURE 5: Isolation and hybridization of abundant informosomal and polysomal cDNAs. (A) Homologous hybridization of informosomal poly(A)$^+$ mRNA with abundant informosomal cDNA [(○) 18 μg/mL RNA]; (---) homologous informosomal curve to $R_0t$ = 0.32 from Figure 4B is shown for comparison. (B) Heterologous hybridization of abundant informosomal cDNA with polysomal poly(A)$^+$ mRNA [(○) 36 μg/mL mRNA, (●) 300 μg/mL RNA, (□) 1.13 mg/mL RNA]; (---) homologous informosomal abundant cDNA curve from panel A is given for comparison. (C) Homologous hybridization of polysomal poly(A)$^+$ mRNA with abundant polysomal cDNA [(○) 18 μg/mL RNA, (●) 180 μg/mL RNA]; (---) homologous polysomal curve to $R_0t$ = 1.0 from Figure 4A is given for comparison. (D) Heterologous hybridization of abundant polysomal cDNA with informosomal poly(A)$^+$ mRNA [(○) 18 μg/mL RNA, (●) 180 μg/mL RNA]; (---) homologous polysomal abundant cDNA curve from panel C is given for comparison.

polysomal and informosomal mRNAs, the nature of which some predictions can be made. As discussed above, the data in Figure 4C indicate that some informosomal mRNA sequences must be represented in the polysomal mRNA with a lower relative frequency. The data in Figure 4D suggest that these informosomal sequences most probably are within the most abundant informosomal mRNA component. These predictions were tested in the experments described below.

*Fractionation and Hybridization of Abundant Informosomal and Polysomal cDNA.* The cDNAs complementary to the most abundant informosomal and polysomal mRNAs were obtained by homologous hybridizations to $R_0t$ values of 0.32 and 1.0 for informosomal and polysomal mRNA, respectively. Following hybridization, the reaction mixtures were treated with $S_1$ nuclease as described under Experimental Procedures with the exception that native calf thymus DNA was omitted from the nuclease buffer. After nuclease digestion, cDNA was recovered by using the same techniques

as those described for the initial synthesis of cDNA.

Abundant informosomal cDNA was hybridized to informosomal mRNA with the result shown in Figure 5A. Hybridization of unfractionated informosomal cDNA is shown for comparison. Approximately 85% of the hybridizable cDNA had annealed at $R_0t$ = 0.32. This same cDNA was also annealed in a polysomal mRNA driven reaction (Figure 5B). It will be noted from Figure 5B that (1) all of the cDNA could again be driven into hybrid form and (2) hybridization was slower than in the homologous reaction. Comparison of the $R_0t_{1/2}$ values of the homologous and heterologous reactions (Figure 5B) indicates that abundant informosomal mRNA sequences are represented in the polysomal mRNA with a considerably reduced frequency (approximately fivefold reduction in frequency). It should be noted that the magnitude of this frequency change is only an estimate. Inspection of the data in Figure 5B shows that abundant informosomal cDNA annealed to polysomal mRNA with nearly ideal pseudo-first-order kinetics. However, hybridization of abundant informosomal cDNA with informosomal mRNA deviated some from that expected for an ideal first-order reaction. Thus, our estimated frequency change may be low for some sequences and high for others, but it is, in any event, very close to that predicted from the data in Figure 4C and Table I. In addition, it should be pointed out that the fivefold reduction in frequency with which the most abundant informosomal mRNAs are represented in polysomal mRNA is a relative number and does not measure the actual partitioning of these mRNAs between the polysomal and informosomal fractions in the cytoplasm. Taking into account the relative frequency of these abundant mRNAs in informosomal and polysomal populations, and the fact that 68% of the mRNA isolated from the cytoplasm is polysomal, it can be calculated that 70% of these sequences are in informosomes and 30% are in polysomes ($0.32 \times 5 = 1.6$ for informosomal mRNA and $0.68 \times 1 = 0.68$ for polysomal mRNA, or 70 and 30%, respectively).

We also compared the relative distribution of abundant polysomal mRNA sequences in informosomal mRNA. Abundant polysomal cDNA was hybridized to polysomal mRNA (Figure 5C). Hybridization of unfractionated polysomal cDNA is shown for comparison. It will be noted that hybridization occurred with nearly ideal first-order kinetics and that ~75% of the hybridizable cDNA had annealed at $R_0t$ = 1.0. This abundant polysomal cDNA was also annealed in an informosomal mRNA driven reaction (Figure 5D). Again, all of the cDNA could be driven into hybrid form. While the $R_0t_{1/2}$ values of the homologous and heterologous reactions were experimentally indistinguishable, the heterologous curve was considerably broader. For instance, some of the abundant polysomal cDNA hybridized faster to informosomal mRNA than to polysomal mRNA. Such a result is consistent with the results presented above. That is, the most abundant informosomal mRNAs, comprising ~75 sequences, are represented in the polysomal mRNA at one-fifth the relative concentration (Figure 5B). This in itself would mean that these abundant informosomal mRNA sequences are diluted in the polysomal mRNA to ~375 sequences and should thus be found in the abundant polysomal mRNA fraction (Table I, Figure 4C). Therefore, it is not surprising that informosomal mRNA can drive some, but not all, of the polysomal abundant cDNA into hybrid form faster than can polysomal mRNA itself.

## Discussion

To date, very little information has been obtained on the diversity of polysomal and informosomal mRNA, and to our knowledge no information is available on the complexity of informosomal mRNA. We have attempted to address both of these questions by using the technique of mRNA–cDNA hybridization. The validity of conclusions drawn from analysis of kinetics of hybridization of cDNA copies of mRNA to vast excesses of template RNA is of course subject to the limitations of the technique. These limitations have been discussed at length (Axel et al., 1976; Hastie & Bishop, 1976), and we will not dwell on them here. That our estimates of sequence complexity of poly(A)$^+$ mRNA (Table I) are within the range of values reported by others (Ryffel & McCarthy, 1975; Jacquet et al., 1978) suggests that we have encountered no undue difficulties in our experiments. Thus, within the constraints imposed by the techniques used, we find that polysomal and informosomal mRNAs have essentially the same sequence complexity.

The diversity of mRNA species in polysomal and informosomal mRNA is still open to question. Levy W & Rizzino (1977) found that a significant portion of the cytoplasmic mRNA from *Drosophila* tissue culture cells was sequestered in the ribosome-free fraction, while Goldstein (1978) reported that substantially the same sequences were represented in the ribosome-free and ribosome-bound fractions of *Drosophila* oocytes and embryos. The diversity of mRNA species in Chinese hamster cytoplasmic polysomal and informosomal mRNA was studied by heterologous annealing reactions with selected cDNAs. We found that (1) essentially all of the cDNA copied from total cytoplasmic mRNA (polysomal + informosomal mRNA) could be driven into hybrid form with either polysomal or informosomal mRNA, albeit with somewhat different kinetics (parts B–D of Figure 3), and (2) all the cDNA copied from either polysomal or informosomal mRNA could be driven into the hybrid form in heterologous reactions (parts C and D of Figure 4 and parts B and D of Figure 5). Thus, essentially all of the mRNA sequences detected were present in both the polysomal and informosomal mRNA. We found no evidence for measurable sequestering of mRNA species in either the polysomal or informosomal mRNA fractions.

While the polysomal and informosomal mRNA shared the same sequences, the two populations were demonstrably different in other respects. Informosomal mRNA contained some sequences present at a considerably higher relative frequency than any seen in polysomal mRNA (compare parts A and B of Figure 4), and the data suggested that the abundant polysomal and informosomal mRNAs were different from each other (Figure 4D) in a manner similar to that reported for abundant liver and kidney mRNA sequences (Hastie & Bishop, 1976). This difference was confirmed in experiments using fractionated cDNA complementary to abundant informosomal and polysomal mRNAs (Figure 5). We conclude that the most abundant informosomal mRNA species are present in the polysomal mRNA with an approximately fivefold reduction in frequency. This is quite different from findings in *Drosophila* tissue culture cells where the concentrations of sequences from the abundant class were similar for both informosomal and polysomal mRNAs (Levy W & Rizzino, 1977).

Our data would seem to put constraints on the role informosomes play in control of gene expression at the translational level. To the extent that cells growing exponentially in culture can serve as a model, it seems unlikely that control is exerted by the complete (or nearly so) sequestering of a considerable number of mRNA sequences in an untranslatable

form in the cytoplasm. Translational control RNA (Bester et al., 1975; Heywood & Kennedy, 1976; Slegers et al., 1977) and association of specific proteins with informosomal mRNA (Gander et al., 1973; Liautard et al., 1976; van Venrooij et al., 1977) have been postulated to serve a role in translational control, but in our system at least such modes of control most probably would not result in complete sequestering of mRNA species. At best, such mechanisms would function in modulation of the distribution of sequences into polysomal and informosomal mRNA in the cytoplasm.

There is in fact reason to believe that mechanisms exist, whatever their exact nature, for precise control of the amount of specific mRNA associated with ribosomes. In reticulocytes, $\beta$-globin mRNA is found almost exclusively in polysomes (Tse & Taylor, 1977; Olsen et al., 1972) while a considerable amount of $\alpha$-globin mRNA is in informosomes (Olsen et al., 1972; Jacobs-Lorena & Baglioni, 1972). In those instances where it is necessary for the cell to very precisely coordinate the synthesis of specific proteins on messengers with differential rates of initiation on the polysome or preferential affinity for initiation factors (Lodish, 1974), the ratio of free to ribosome-bound mRNP may be very important. The higher relative frequency of some mRNA sequences in informosomes that we report here may be one way in which the cell ensures itself of an appropriate supply of particular mRNAs. That is, mRNAs required in polysomes in the same abundance as mRNAs having much greater affinities for initiation factors could achieve their equivalent polysomal abundance by being more abundant in informosomes.

Although we favor the above view that differential apportioning of some mRNA species between ribosome-free and ribosome-bound populations in the cytoplasm plays a positive role in coordinating the synthesis of specific proteins, other explanations are possible. For instance, it may be that a portion of the chains of a particular mRNA species is permanently in an untranslatable state in the cell. In addition, we cannot exclude the possibility that cell cycle associated differences in the metabolism of polysomal and informosomal mRNAs (Enger et al., 1974; Enger & Campbell, 1975) play a role in their cytoplasmic distribution; e.g., a full complement of mRNA chains of a given species may be active in some cells and inactive in others.

The salient question we addressed in this study is whether a cytoplasmic population of mRNA species exists solely in an untranslatable form awaiting, perhaps, the appropriate stimulus to become associated with ribosomes (an analogy of the situation which exists in the fertilized egg). We did not find this to be true for exponentially growing Chinese hamster cells. As discussed above, however, different cell types and growth states may yield different results. Thus, it is clear that this problem must be studied in a number of different systems, using cDNA as a probe not only for the total mRNA population but also for the single mRNA species as well. Such studies should provide insight into the role informosomes play in control of gene expression in cells with established and essentially unchanging patterns of gene expression as well as in those cells in which such patterns are changing coordinately with development, differentiation, or in response to changing external effectors.

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