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Chinese Hamster Polyadenylated Messenger Ribonucleic Acid: Relationship to Non-Polyadenylated Sequences and Relative Conservation During Messenger Ribonucleic Acid Processing

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We have further analyzed the metabolism of specific messenger ribonucleic acid (mRNA) sequences within the cytoplasmic and nuclear RNA of Chinese hamster ovary (CHO) cells by using a set of previously constructed complementary deoxyribonucleic acid (DNA) clones (Harpold et al., Cell 17:1025-1035, 1979) as specific molecular probes in a variety of RNA:DNA hybridization experiments. The majority of the labeled mRNA complementary to each of the nine clones was found in the polyribosomes, with some variation between individual sequences. The great majority of each specific mRNA labeled for 3 h or less was in the polyadenylated [poly(A)$^+$] fraction. However, the amount of each sequence increased in the non-poly(A)$^+$ [poly(A)$^-$] fraction after very long label times, suggesting the derivation of the poly(A)$^-$ RNA from the poly(A)$^+$ RNA. Eight of the nine mRNA's have cytoplasmic half-lives ranging from 8 to 14 h, whereas one of the mRNA's, the scarcest in the group, has a somewhat shorter half-life of approximately 3 h. The proportion of each of the specific long-lived mRNA's within the total labeled mRNA increased as a function of labeling time, indicating that a large fraction, probably greater than 50%, of the initially labeled poly(A)$^+$ mRNA in CHO cells has a half-life of less than 3 h. A quantitative analysis of the kinetics of labeling of specific nuclear and cytoplasmic sequences indicated that a significant fraction of the mRNA sequences transcribed from genes containing these nine CHO sequences were successfully processed into mRNA. However, two of the CHO mRNA sequences were only partially conserved during nuclear processing to yield mRNA. These studies demonstrated that events at two post-transcriptional levels, differential nuclear processing efficiency of different primary transcripts and cytoplasmic stability of different mRNA's, can be involved in the determination of the cytoplasmic concentrations of different mRNA's.

The study of total cell ribonucleic acid (RNA) from cultured cells has provided numerous suggestions, but no definitive conclusions, about many phases of messenger RNA (mRNA) metabolism (5, 6). For example, based on the average ultraviolet target size, most mRNA molecules in HeLa cells and L cells appear to derive from larger heterogeneous nuclear RNAs (hnRNA's) (7-9). The majority of total mRNA in polysomes from a wide variety of cells appears to contain a 3'-terminal polyadenylic acid [poly(A)] segment (1, 3, 11, 20). Among specific mRNA's, only histone mRNA in mammalian cells appears certain to arrive in the cytoplasm lacking poly(A) (1, 3). According to some reassociation kinetic experiments, however, a large part of the sequence complexity of the polyadenylated [poly(A)$^+$] fraction is different from that found in the non-poly(A)$^+$ [poly(A)$^-$] fraction (10, 32), whereas other studies suggest almost total overlap in sequence complexity (14) between poly(A)$^+$ and poly(A)$^-$ sequences. Moreover, translation of the poly(A)$^-$ mRNA shows considerable overlap between the two fractions (11, 18-20). A third general problem in mRNA metabolism, mRNA turnover, has also been explored by studying total cell mRNA. A large fraction of cultured cell mRNA has a long half-life (5 to 15 h) (12, 23, 31), but there is kinetic evidence that suggests a substantial fraction with short half-lives (24). These problems in mRNA metabolism—identification of a primary transcript, efficiency of hnRNA processing, and cytoplasmic distribution and stability—must be addressed effectively to pinpoint the level of control for specific genes.

The use of pure segments of deoxyribonucleic acid (DNA), either virus DNA or recombinant DNA, to hybridize specific mRNA's is the key to providing more complete answers to the prob-

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lems of mRNA metabolism. We began our studies of the metabolism of specific Chinese hamster cell (CHO cell) mRNA's (13) by preparing a set of so-called "cDNA clones," plasmid DNAs that contain partial DNA copies of individual poly(A)+ cytoplasmic RNAs (15). With these clones we were able to measure the rate of nuclear RNA synthesis and the cytoplasmic concentration of each of nine individual mRNA species. For those RNAs that were synthesized fast enough to be significantly labeled with a brief pulse, we could show specific, higher-molecular-weight nuclear RNA that was presumably the precursor for each of these cytoplasmic mRNA's (13).

In this paper, using the same cDNA clones, we further characterize the cytoplasmic and nuclear RNA of Chinese hamster cells in a variety of ways. The poly(A)+ cytoplasmic RNA complementary to these cloned segments is mostly in the polyribosomes, and almost all of the newly labeled specific mRNA is in the poly(A)+ class. After very long label times a much larger fraction of the RNA complementary to the cloned sequences was in the poly(A)- class.

The cytoplasmic half-lives of the poly(A)+ mRNA's were established for each of the specific nine mRNA's and ranged from 3 h for the scarcest mRNA to 14 h. At least half of the total newly arrived mRNA molecules turn over considerably faster than 3 h, based on the rising percentage of the total represented by specific mRNA sequences.

In the accompanying paper (27) we show that over 90% of the newly formed caps in polysomal RNA larger than histone mRNA are in the poly(A)+ mRNA class. Considering these findings together with the results in this paper, we conclude that the large number of newly labeled capped, poly(A)- cytoplasmic molecules are not the same sequences as the poly(A)+ RNA.

We have also studied the total accumulation curves of nuclear and cytoplasmic RNA complementary to seven of the nine cloned DNA sequences that hybridize enough pulse-labeled RNA to accurately measure. The accumulation of specific labeled RNA suggests that a significant fraction of each species of labeled nuclear RNA is conserved in making mRNA. However, one or perhaps two of the specific sequences accumulate in the cytoplasm at a slower rate relative to the nuclear accumulation rate than the other five specific sequences. Since the cytoplasmic half-life of all these species is quite long (8 h or more), it appears that some of these specific sequences must turn over in the nucleus.

MATERIALS AND METHODS

Cell growth, labeling conditions, and RNA isolation. The growth of suspension cultures of CHO cells, labeling of the cells with [^{3}H]uridine, and isolation of cytoplasmic and nuclear RNA have been described previously (13). Specific labeling conditions are detailed in the legends to the tables and figures. The fractionation of those RNA molecules containing poly(A), the poly(A)+ fraction, from the remainder of the poly(A)- fraction was carried out by Sepharose chromatography. The conditions were chosen so that molecules bearing poly(A) segments of 20 to 30 or more residues would be retained in the poly(A)+ fraction (21, 28, 35). The chromatography was at 25°C, binding was in 0.2 M NaCl–0.2% sodium dodecyl sulfate–0.01 M ethylenediaminetetraacetic acid, and the unbound fraction was eluted from the column in the same buffer with no sodium chloride.

cDNA clone procedures. The preparation and isolation of the nine CHO cDNA clones were performed as described previously (13) in accordance with the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules. The clones are designated A through I throughout the paper.

Hybridization procedures. Labeled RNA was hybridized to an excess of filter-bound cDNA plasmids and measured as described (13).

RESULTS

Presence of nine specific sequences in the polysomes of CHO cells and distribution in poly(A)+ and poly(A)- categories. Our previous report (13) did not present evidence that the poly(A)+ RNAs complementary to the nine cloned DNA sequences from CHO cells were found in polyribosomes, nor did we examine the distribution of the specific RNAs in poly(A)+ compared to poly(A)- fractions. To investigate both these points, growing CHO cells were labeled with [^{3}H]uridine for 3 h, and the poly(A)+ RNA was prepared from polyribosomes and non-polyribosomal cytoplasmic fractions. (The division of the polyribosome gradient was at 90 to 100S or approximately the sedimentation position of a two-ribosome peak.) Approximately 60% of the total labeled poly(A)+ RNA was recovered in polyribosomes (Table 1), a distribution similar to that of capped molecules described in the accompanying paper (27) and similar to the total distribution of poly(A) segments described for cultured human cells some years ago (16). The majority of the newly labeled mRNA complementary to each of the nine cloned sequences was found in the polyribosomes (Table 1), but variation was observed between the individual sequences. For example, 80% or more of the RNA complementary to clones A, C, G, and H was in polyribosomes, but only 40 to 50% of the RNA complementary to clones F and I was in polyribosomes.

The distribution of specific mRNA's in the poly(A)+ and poly(A)- fractions of cytoplasmic RNA was determined in a number of experiments (Tables 1 and 2). Both in the polysomal

TABLE 1. *Distribution of specific CHO sequences in polysomes*[a]

Clone	Polysomal (>100S)			Nonpolysomal (<100S)		
	Poly(A)$^+$ (cpm)	Poly(A)$^-$ (cpm)	% in >100S fraction	Poly(A)$^-$ (cpm)	Poly(A)$^-$ (cpm)	% in <100S fraction
Total	2.4×10^6		60	1.6×10^6		40
A	2,350	400	83	440	120	17
B	3,600	520	60	2,300	420	40
C	2,015	935	84	390	175	16
D	250	35	70	110	15	30
E	1,240	615	73	467	225	27
F	670	99	38	1,160	90	62
G	710	90	84	109	40	16
H	725	105	80	142	70	20
I	345	110	49	360	110	51

[a] Cytoplasmic RNA from cells labeled with [^{3}H]uridine for 3 h (150 μCi/ml, 20 mCi/mol; final uridine concentration, 0.01 mM) was prepared by separating cytoplasm into polysomal fraction (>100S) and nonpolysomal fraction (<100S), extracting the RNA from each, separating poly(A)$^+$ from poly(A)$^-$ RNA by polyuridylic acid-Sepharose chromatography, and hybridizing the RNA from each fraction to an excess of nitrocellulose-bound DNA from each cloned species. The DNA excess was assured by rehybridizing the supernatants to be sure that >80% of specific RNA was removed during the original hybridization (see reference 13). This experiment in its entirety was performed only once, but on other occasions specific sequences have been found to be from 50 to 80% in polyribosomes.

TABLE 2. *Percentage of specific CHO mRNA's in poly(A)$^+$ fraction after various label times*[a]

Cloned DNA	% in poly(A)$^+$ fraction after label times:				
	30 min	60 min	90 min	150 min	210 min
A	89	91	92	80	84
B	94	92	94	91	85
C	75	94	92	90	70
D	—	—	80	76	87
E	95	91	91	84	65
F	—	91	92	89	91
G	—	87	93	95	86
H	—	95	96	97	83
I	—	—	96	98	75

[a] After various labeling periods, the total cytoplasmic RNA was extracted, separated into poly(A)$^+$ and poly(A)$^-$ fractions by polyuridylic acid-Sepharose chromatography, and hybridized to an excess of each specific DNA bound to nitrocellulose. Figures entered in this table were from experiments where at least 300 total cpm was hybridized in the poly(A)$^+$ sample to each cloned DNA sample. The actual amount hybridized ranged from 300 to 15,000 cpm, depending on the abundance of the RNA species.

RNA and in the postpolysomal RNA, the great majority of the newly labeled RNA complementary to each clone was in the poly(A)$^+$ fraction, indicating that, in this majority, any newly labeled poly(A)$^-$ cytoplasmic RNA sequences could not simply be the same RNAs without poly(A). Specifically, the large amount of newly synthesized capped poly(A)$^-$ molecules described in the accompanying paper (27) are a different class from the specific poly(A)$^+$ polysomal molecules.

However, the percentage of each specific cloned sequence present in the poly(A)$^-$ category increased with longer label times, so that from 20 to 45% of the total specifically hybridized RNA was in the poly(A)$^-$ class at steady state (Fig. 1). The poly(A)$^+$ fractionation was carried out on all these RNA preparations in such a way that over 90% of molecules having 20 to 30 or more adenylic acid residues on the 3′ terminus would have been retained in the poly(A)$^+$ class (28, 35). Thus, the poly(A)$^-$ fraction contains no (or very few) newly made sequences of the poly(A)$^+$ variety but significant amounts of these sequences as old molecules, some of which derive from the poly(A)$^+$ fraction presumably by slow loss of poly(A). It appears that the poly(A)$^-$ group of molecules is not a homogeneous set of molecules distinct from the poly(A)$^+$ molecules, but a heterogeneous group of molecules. At present the only polysomal mRNA's of the cell known to arrive in the cytoplasm without poly(A) are the histone mRNA's (1, 3).

Choice of the technique to study the metabolism of mRNA sequences. With the availability of pure DNA sequences complementary to a set of specific mRNA molecules, it is possible to directly measure the accumulation of specific labeled RNA sequences first in the nucleus and then in the cytoplasm and thus to chart the metabolic fate of specific mRNA sequences. We have used this technique to study the metabolic fate of nuclear transcripts and cytoplasmic stability of adenovirus-specific mRNA's and have detected different mRNA

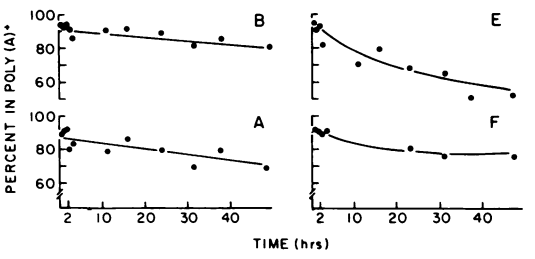

FIG. 1. *Percentage of specific CHO mRNA's in poly(A)+ fraction at approach to steady state. Cells were labeled and mRNA was prepared and hybridized to specific DNA sequences as described in Tables 1 and 2 and Fig. 2. The proportion of each of four specific sequences (A, B, E, and F) in the poly(A)+ fraction from early label times to later label times is given.*

stabilities for different mRNA's in both infected and transformed cells (4, 33–35). The technique relies on the fact that within 10 to 20 min a maximum specific activity of the uridine 5′-triphosphate pool is established (24). The accumulation of labeled uridine 5′-monophosphate residues in specifically hybridized mRNA (which is a function of the rate of turnover, assuming synthesis to be constant) can be easily measured. Any inaccuracy of this technique occurs because: (i) the pool takes a finite time to become radioactive; (ii) labeled nuclear RNA requires some processing time to yield labeled cytoplasmic mRNA; and (iii) both uridine 5′-monophosphate and cytidine 5′-monophosphate become labeled, but the latter lags behind the former by 30 to 60 min. Corrections for all of these factors can be easily done, and the inaccuracies are approximately ±15 min. Only the estimation of rapidly turning over mRNA species is substantially affected by these corrections. Moreover, the "accumulation" technique for measuring mRNA half-lives is metabolically innocuous, whereas many "chase" techniques use conditions in which cells can no longer grow and that conceivably can change the half-life of the mRNA. For example, we found that actinomycin treatment of previously labeled transformed cells allows an accurate half-life estimation of the adenovirus mRNA (31, 32). However, early in infection the turnover of the same two mRNA's cannot be measured accurately during an actinomycin chase (33). In addition, the inherent difficulty of pulse-chase experiments, i.e., that a chase is never immediately or completely effective (24), is avoided. If a chase is too slow, then the half-life of rapidly turning over species appears to be too long, and long-lived species appear even more long lived.

We therefore chose to study the metabolic stability of the nine specific CHO mRNA's by the accumulation of labeled uridine 5′-monophosphate into mRNA in growing suspension cultures of Chinese hamster cells. Cells were exposed to [^{3}H]uridine at a specific activity and total uridine concentration that allowed continued growth for at least three generations without exhausting the labeled uridine in the medium (see legend to Fig. 2). The poly(A)+ mRNA was collected at intervals after the beginning of labeling, and the amount of radioactivity in each mRNA was measured by hybridization (Fig. 2). (Corrections for cell growth and for conversions to cytidine 5′-monophosphate were made at each time point [22].) From the time it took to reach a plateau value for the label within each specific mRNA, a half-life for each mRNA could be calculated (12). A best-fit curve matched to the data for each mRNA, as well as half-life curves that were either shorter or longer than the best fit, were calculated (Fig. 3). This analysis suggested that the confidence of the estimation of half-lives was at least ±40% (Fig. 1). Eight of the nine mRNA's had half-lives estimated to be between 8 and 14 h. Although it is not possible within the accuracy of the measurements to be certain of the exact differences between different mRNA species, it is clear that not all the longer-lived species have identical half-lives. In addition, the scarcest mRNA in the group, the one complementary to clone I, has the shortest half-life, estimated to be about 3 h.

The nine clones used in this work were selected from 50 original clones by determining the percentage of input [^{3}H]mRNA (3-h label) that hybridized to each cloned DNA sample (13). These nine clones hybridized a larger fraction of the total mRNA than did most of the 40 clones that were not included for study. Thus, since eight of these nine mRNA's have long half-lives, it is possible that, in general, the longer-lived mRNA's are those that constitute a larger fraction of the mass of the cell mRNA.

If the percentage of the total labeled mRNA complementary to each clone is plotted as a function of labeling time, several useful conclusions result (Fig. 4). First of all, during the first 60 to 90 min of labeling the concentration of each mRNA as a fraction of the total labeled mRNA remained approximately constant. Each of the nine species of mRNA therefore appears, on the average, to be delivered equivalently to the cytoplasm together with the general population of mRNA. With time, however, the long-lived mRNA's rose to higher proportions of the total mRNA. This presumably reflects the greater dilution at early label times of newly labeled long-lived mRNA by short-lived mRNA. mRNA's with the longest half-life (Fig. 4, B and F) increased in concentration by fivefold between 1 and 36 h, and the mRNA's with the next

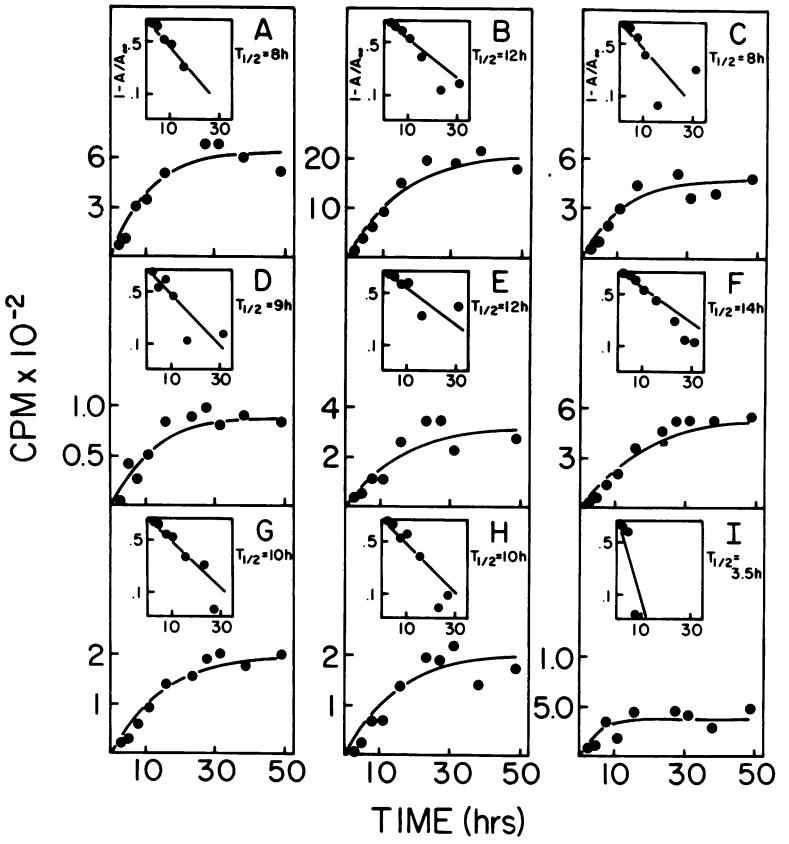

FIG. 2. *Determination of half-life of specific CHO mRNA molecules by accumulation of labeled mRNA. Growth medium with [^{3}H]uridine (22.5 Ci/ml; uridine concentration, 0.02 mM) was prepared, and cells were suspended at 37°C in this medium. Samples were removed from 1.5 h through 55 h. The culture was diluted with the same labeled medium twice during the experiment. Cytoplasmic RNA was extracted from an equal volume of culture at each indicated time, and the optical density of the total RNA at 260 nm was determined. Based on the accumulation of RNA, the cells grew exponentially with a doubling time of 17 h throughout the experiment. The poly(A)$^+$ cytoplasmic RNA was isolated from each sample and hybridized to excess filter-bound DNA of each of the nine CHO cloned sequences. The counts per minute hybridized was corrected for the cell number (based on the increase in total cytoplasmic optical density at 200 nm), and the half-life was estimated according to the equation of Greenberg (12). The inserts show the reciprocal plots obtained from each accumulation curve.*

longest half-life increased by a factor of three to four (A, C). Thus about three-fourths of the total newly made mRNA had a half-life shorter than these long-lived mRNA's. Perhaps more surprising was the finding that the concentration of mRNA complementary to clone I, an mRNA that has a half-life of about 3 h, also increased by a factor of 2.5 between 1 and 6 h of labeling. Thus more than one-half of the total mRNA to reach the cytoplasm turns over with an average half-life considerably shorter than 3 h. These results give direct support to the earlier conclusion that a substantial fraction of mRNA of cultured mammalian cells has a rapid half-life (24).

Although we do not have a cloned CHO sequence complementary to an RNA with a very short half-life, we made the same measurements shown in Fig. 4 for an mRNA in another cell type. Rat embryo cells, strain 8617, transformed by adenovirus type 2, have adenovirus DNA integrated into the cell DNA (28). These cells produce two types of mRNA's whose half-lives are about 35 and 100 min, respectively (33–35). [^{3}H]uridine accumulation into the total group of adenovirus mRNA's has been measured in many experiments, and the data are plotted in Fig. 5 in the same fashion as the data for the specific CHO mRNA's in Fig. 4; that is, the percentage of the labeled mRNA that was adenovirus spe-

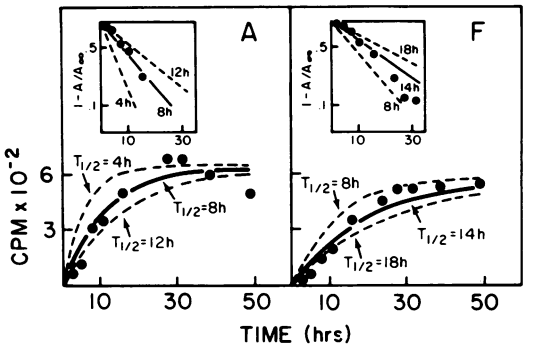

FIG. 3. *Accuracy of mRNA half-lives from accumulation curves. The accumulation data obtained as described in Fig. 1 for two mRNA's (A and F) are plotted in comparison with computer-derived curves showing three different half-lives. It appears that the half-life estimations derived from the data (Fig. 1) are accurate to at least ±40%.*

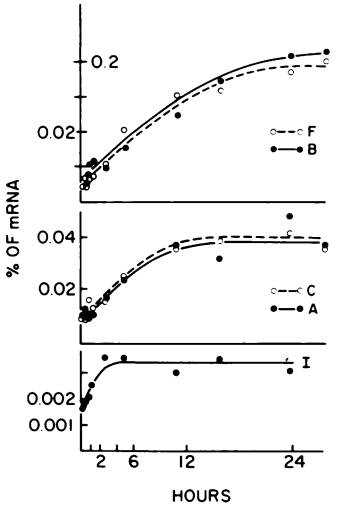

FIG. 4. *Change in population of labeled mRNA's as a function of label time. The data from the experiment shown in Fig. 2 were plotted to show, as a function of label time, the percentage of poly(A)+ RNA that was complementary to each of five of the specified cloned DNA sequences. (Data for clones A, B, C, F, and I are shown.)*

cific is plotted as a function of label time. In this case, presumably because the mRNA is rapidly turned over, there is a higher percentage of the total labeled mRNA that is adenovirus specific after short label times, which declines as more stable mRNA's accumulate. These results are in accord with the conclusions that most (over half) of the poly(A)+ mRNA of CHO cells has an average half-life considerably less than 3 h.

Accumulation of labeled specific nuclear sequences compared to specific cytoplasmic sequences. With a measurement of cytoplasmic half-lives for each of the nine CHO mRNA's, attention was turned to the question of conservation of hnRNA sequences during processing; that is, does each primary nuclear RNA transcript give rise to an mRNA? By examining the rate of accumulation of specific sequences in the nucleus followed by the rate of cytoplasmic appearance of those same sequences, this question can be at least partially evaluated. The accumulation of labeled specific sequences in nuclear and in poly(A)+ cytoplasmic RNA in two different experiments is given in Fig. 6. In all cases the amount of specific cytoplasmic RNA exceeded that in the nucleus within 60 to 90 min, indicative of significant conservation of each sequence in the manufacture of mRNA. For example, in many older experiments the label in total hnRNA was found to exceed the amount of poly(A)+ mRNA for many hours (16, 17), indicative of a lack of conservation of the majority of nuclear RNA sequences.

A closer inspection of the amount of labeled RNA complementary to clone A compared, for example, to clones B or C shows an interesting quantitative difference. After the shortest label time, slightly more labeled nuclear RNA hybrid-

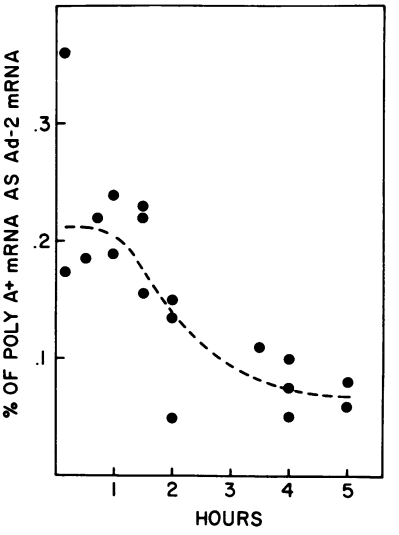

FIG. 5. *Change in proportion of labeled adenovirus-specific mRNA in transformed cells. Adenovirus-transformed rat embryo cells, strain 8617, were labeled with [^{3}H]uridine (100 to 200 μCi/ml, 20 mCi/mmol) for various times, and poly(A)+ cytoplasmic RNA was prepared. The fraction of the radioactive RNA complementary to adenovirus DNA between 0 and 11 (the 3,500 bases on the "left" end of the adenovirus genome; see references 30–32) was determined.*

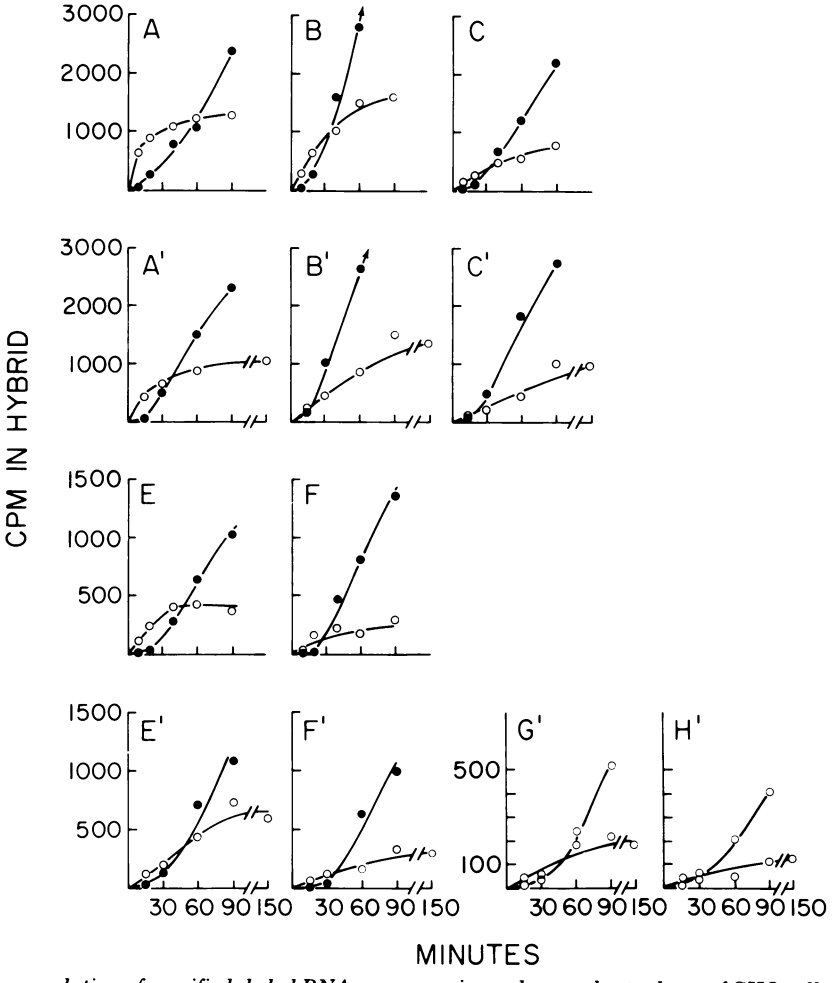

FIG. 6. *Accumulation of specific labeled RNA sequences in nucleus and cytoplasm of CHO cells. CHO cells were labeled with [^{3}H]uridine (200 μCi/ml; 0.01 mM final concentration) for times indicated. The total nuclear (●) and cytoplasmic (○) RNA were hybridized to an excess of each of the specific DNA sequences.*

ized to clone A than to clone B, indicating a slightly faster rate of synthesis of A sequences compared to B sequences. Furthermore, at steady state there was about an equal amount of A and B sequences in the nucleus. However, within the first 150 min of label time twice as much labeled B RNA sequences appeared in the cytoplasm as labeled A sequences.

To compare the accumulation data for a variety of specific sequences, the data from three separate accumulation experiments were plotted in a normalized form. The labeled nuclear RNA for each specific sequence was observed to plateau between 60 and 90 min (Fig. 7), and this plateau value was taken as 1.0; the relative amount of labeled nuclear and cytoplasmic RNA at each time point was then normalized to this value. This allowed a comparison of the efficiency of production of cytoplasmic RNA for seven of the nine available cloned sequences. (The amount of labeled RNA that hybridized to clones D and I before 60 min was so low they were not included in this analysis.) For five of the seven specific sequences (B, C, F, G, and H) the amount of cytoplasmic RNA was 1.5 to 2.5 times that of the nuclear plateau by 60 min of labeling, 2 to 4 times greater by 90 min of labeling, and 4 to 7 times greater by 150 min of labeling. In every experiment the relative accumulation of cytoplasmic RNA complementary to clones A and E was about half that for the other five clones. This was true in spite of the fact that the hybridizable nuclear RNA specific for both clones A and E reached a plateau just as rapidly as that for the other specific sequences (Fig. 6), arguing against a relatively larger nu-

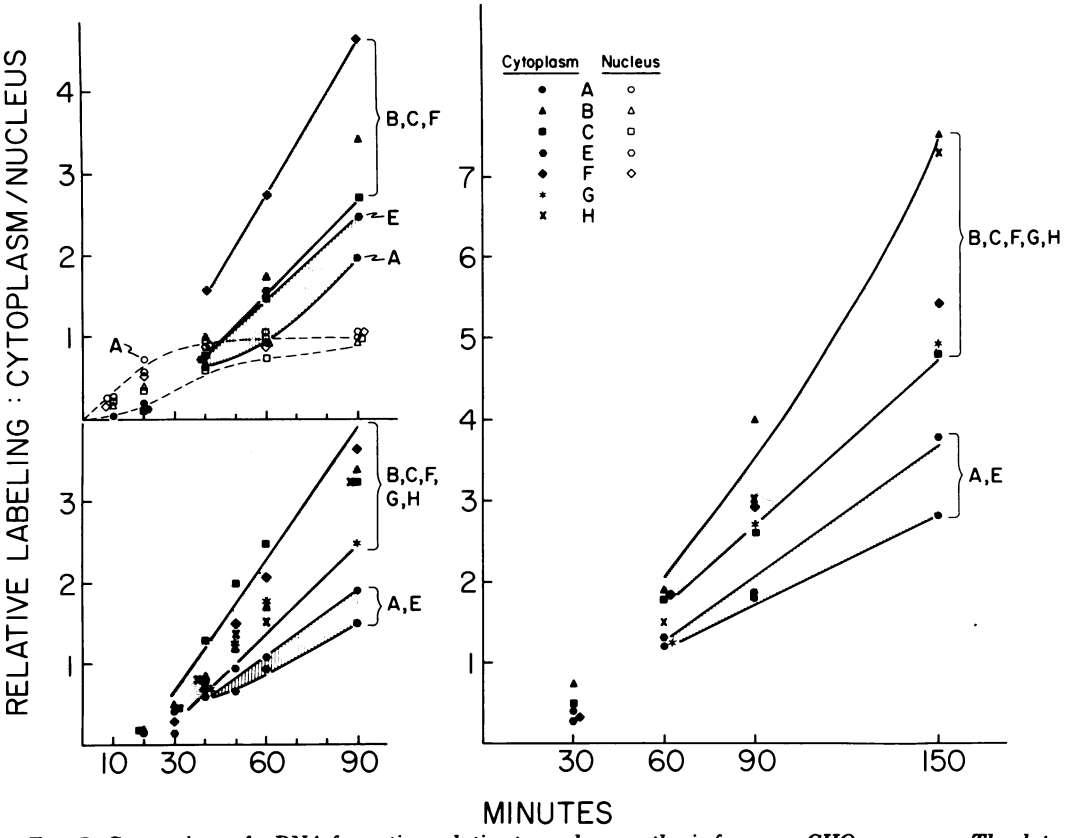

FIG. 7. *Comparison of mRNA formation relative to nuclear synthesis for seven CHO sequences. The data from three [^{3}H]uridine accumulation experiments (performed as described in Fig. 5) were normalized to allow comparison of the relative efficiency of accumulation in the cytoplasm compared to the nucleus for each of seven specific sequences. A plateau value of labeled nuclear RNA for each cloned sequence was taken as 1.0; the hybridized labeled RNA from each point on the accumulation curve for each specific sequence was normalized to this value and plotted. The shaded areas represent the five clones whose nuclear sequences yielded the highest amount of mRNA, and the hatched areas show that nuclear RNA complementary to clones A and E consistently yielded relatively less cytoplasmic mRNA.*

clear pool for A and E sequences than for the other five sequences. In addition, the A and E mRNA's have long cytoplasmic half-lives, so the relatively lower rate of cytoplasmic accumulation of these two mRNA's could not be caused by a more rapid cytoplasmic turnover. Rather it appeared that for these two of the seven specific sequences under study, cytoplasmic RNA was produced relatively less efficiently from nuclear transcripts. We next attempted a quantitative mathematical analysis of the accumulation data.

We have previously described how to assess accumulation data of labeled specific RNA sequences in order to estimate the processing efficiency (Materials and Methods; 4, 22). The three determining factors in the rate of nuclear appearance and then cytoplasmic accumulation of any given mRNA sequence are (i) rate of nuclear synthesis, (ii) the processing time and efficiency of processing, and (iii) the cytoplasmic turnover. Since we have experimentally measured the relative (not absolute) rates of synthesis of the nine cloned sequences as well as the cytoplasmic turnover times (this paper), computer curves can be drawn of expected label accumulation for a given sequence. Three parameters are varied: estimated actual rate of synthesis (in counts per minute per minute), the nuclear processing time, and the efficiency of processing. From the fit of the computer curves to the experimental accumulation data an estimate was made of the efficiency of nuclear processing for the separate CHO sequences (Fig. 8). From this analysis of the accumulation data it was suggested that as much as 75% of the nuclear RNA complementary to A, and 50% of the nuclear RNA complementary to E, might fail to reach the cytoplasm. The specific sequences for

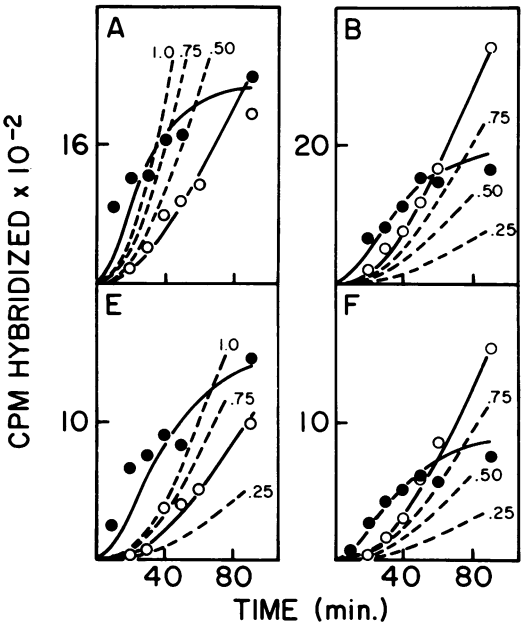

FIG. 8. *Kinetic analysis of label accumulation in specific sequences. Computer-drawn curves are plotted that indicate different levels of conservation in nuclear RNA processing (0.25 = 25% conservation, 0.5 = 50% conservation, etc.). The equations of Chen-Kiang et al. (4) were used in the computations. The experimental values were: (i) relative rate of specific RNA synthesis (this paper, Fig. 6, and reference 13), (ii) uridine 5'-triphosphate pool equilibration half-time of 8 min (measured as described in references 21 and 23) and (iii) cytoplasmic mRNA half-life as in Fig. 2. An absolute rate constant for nuclear RNA accumulation was determined from a computer fit to the accumulation curve for nuclear specific labeled RNA. A composite rate constant that represents "nuclear dwell time" and "nuclear life-time" (see reference 4) was then chosen to fit the accumulation curves. If the composite rate could not include a real fraction (25, 50, or 75%) of nuclear RNA that decayed and still produce an accumulation curve that matched the data, a high level of conservation was indicated. Accumulation patterns for B and F (right side of figure) suggest high conservation, as did the patterns for RNA complementary to C, G, and H. The patterns of RNA complementary to A and E (left panels) did not appear to be so highly conserved.*

all the other CHO mRNA's appeared to exit to the cytoplasm with a high degree of conservation (Fig. 8). This analysis supports the conclusion of Fig. 7 that A and E sequences are not as effectively converted to mRNA as are B, C, D, F, and G sequences.

However, we remain unsure that complete conservation in fact occurs for any of these sequences because of another experimental observation. Since each newly made specific CHO mRNA enters the cytoplasm as a poly(A)$^+$ molecule (Table 2), and since poly(A) appears to be added to nuclear RNA almost instantly after synthesis (22, 26), we should expect all nuclear RNA complementary to one of the cloned sequences to be in the poly(A)$^+$ category if conservation were complete. However, preliminary evidence suggests that a substantial fraction of these specific CHO sequences are in a poly(A)$^-$ nuclear RNA fraction and therefore are not conserved. The evidence is insufficient at present to reach a firm conclusion because of two technical problems: (i) we do not know the location of our cloned sequences within primary transcripts, and (ii) without alkali breakage of hnRNA to an average size of 5,000 bases we cannot select 100% of the nuclear poly(A)$^+$ molecules (21). Therefore we are unsure how much of the specific A to I CHO sequences truly reside in the poly(A)$^-$ nuclear fraction. Genomic clones in which the sequences just next to the poly(A) site are identified will be necessary to settle this question, and these are being prepared. With such genomic clones we could satisfactorily study the presence in poly(A)$^+$ and poly(A)$^-$ molecules of specific RNA sequences that closely neighbor poly(A).

However, the present comparisons in the accumulation curves (Fig. 6 and 7) indicate already that not every nuclear RNA molecule of the A and E type is destined to be processed successfully.

DISCUSSION

In this work we have used a set of specific cloned DNA segments to explore general features of mRNA metabolism in CHO cells. It appears that the RNA complementary to these cloned DNA segments is part of a polysomal, poly(A)$^+$ RNA class, presumably mRNA. Furthermore, the newly labeled poly(A)$^+$ and poly(A)$^-$ cytoplasmic RNAs seem to be distinct, largely nonoverlapping sequence categories. However, there is an increase in the fraction of each specific sequence in the poly(A)$^-$ cytoplasmic RNA after long label times, suggesting derivation of some poly(A)$^-$ RNA from poly(A)$^+$ molecules due to loss of poly(A) (30). Together with the results in the accompanying paper (27), that no significant amount of newly labeled polysomal, poly(A)$^-$ capped molecules exists (other than histone mRNA), these results suggest that no distinct class of poly(A)$^-$ mRNA sequences truly exists. This conclusion has been stated before with varying degrees of firmness (3, 11, 18–20), based on translation results which do not show a distinct category of proteins encoded by poly(A)$^-$ molecules.

With the availability of specific recombinant DNA molecules that can be purified in large amounts, the time course of labeling of the specific RNA sequences in the nucleus and in the cytoplasm can be determined. Eight of the nine mRNA's studied have long cytoplasmic half-lives (8 to 14 h), and one, the scarcest in the group, has a somewhat shorter half-life (3 h). From the changing proportions of the total labeled poly(A)$^+$ mRNA's represented by each specific sequence, it is evident that a large fraction, more than 50% of the total poly(A)$^+$ mRNA, has a half-life considerably shorter than 3 h. These results establish for the first time by direct experiment the existence of such a large fraction of rapidly turned-over cytoplasmic poly(A)$^+$ mRNA and call attention to the likelihood that a continuum of turnover times exists from a few minutes to many hours.

The apparent rapid turnover of a large fraction of the newly labeled cytoplasmic CHO poly(A)$^+$ mRNA is of special interest in relation to the newly labeled capped, poly(A)$^-$ cytoplasmic RNA fraction described in the accompanying paper (27). In those experiments, after a short [*methyl*-^{3}H]methionine label, a greater number of labeled caps were found in the poly(A)$^-$ molecules of mRNA size than in poly(A)$^+$ cytoplasmic mRNA. At steady state the reverse was true, evidence of a rapid turnover of the newly synthesized capped poly(A)$^-$ molecules compared to new poly(A)$^+$ molecules. The results suggest that the newly labeled capped poly(A)$^-$ cytoplasmic RNA fraction has a half-life that could be 1 h or less.

Finally, a quantitative examination of the kinetics of labeling of specific nuclear and cytoplasmic sequences allows the conclusion that a significant fraction of the molecules transcribed from genes containing these nine sequences are in fact successfully processed into mRNA. However, at least one specific sequence (clone A), and perhaps one other (clone F), is conserved only one-quarter to one-half as well as the majority of the sequences during the processing that yields mRNA. One mechanism for only partial conservation of a transcript is illustrated by the processing of adenovirus primary transcripts (22) and the processing of primary transcripts of immunoglobulin heavy-chain genes (2, 25). In those cases there are two or more poly(A) stations in the primary transcript, but only one poly(A) site can be used in each transcript, so some portion of each primary transcript is destined to be discarded in making the mRNA.

Additional work, involving isolation of genomic clones of DNA that contain each of the A and E sequences and a more thorough examination of parts of the primary transcripts next to poly(A), is required to quantitate more exactly the extent of conservation of the specific molecules as poly(A)$^+$ RNA. It is clear from the accompanying paper (27) that only one of three hnRNA molecules receives a poly(A) segment and contributes a polysomal poly(A)$^+$ molecule. Whether the nuclear transcripts that are not successfully processed are extra copies of the same primary transcripts that are successful, or are a totally different class, is not yet clear. As mentioned previously preliminary experiments indicate that a considerable fraction (at least a third and maybe two-thirds) of the sequences complementary to the cDNA clones used here are unattached to poly(A). Until we can definitely settle the issue of how much of the apparent poly(A)$^-$ nuclear RNA makes up the same set of primary sequences as the poly(A)$^+$ molecules, the question of the nature of the "extra" capped large hnRNA that fails to receive poly(A) remains unknown.

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