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RATE OF SYNTHESIS AND CONCENTRATION OF SPECIFIC mRNA SEQUENCES IN CULTURED CHINESE HAMSTER OVARY CELLS COMPARED TO LIVER CELLS

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SUMMARY

With the aid of recombinant DNA molecules the concentration of nine different mRNAs in cultured Chinese hamster cells has been compared to liver cells. One clone in nine showed a marked increase (13-fold) in liver cells but the remainder were present at about the same level in the two cell types. The rate of RNA synthesis in the nucleus was not increased by 13-fold for this clone but only about 3-fold. These data show that quantitative hybridization to recombinant DNA can estimate mRNA concentrations and rates of synthesis accurately and suggest levels at which control operates.

INTRODUCTION

Cross-hybridization of cDNAs synthesized from mRNA sequences isolated from different tissues shows extensive homology in the total mRNA between differentiated cells (1,2,3). Similarly, two-dimensional gel analysis of the total protein content of different cells in the same animal suggests the general conclusion that many proteins are present in similar concentrations in different cell types (4,5). However, the frequency and the extent to which individual mRNA or protein concentrations vary is hard to quantitate by these two methods. We have recently prepared *E. coli* plasmids containing segments of DNA complementary to nine randomly chosen mRNA molecules from Chinese hamster cells and used this DNA to score the concentration of each mRNA (6). We have also estimated the transcription rate for sequences complementary to each of these nine clones. In the present work we compared the concentration of these specific sequences in Chinese hamster liver cells to the concentration in CHO cells.

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This initial approach supplies a specific and quantitative measurement essential to the problem addressed above, namely: How constant are the rates of synthesis and the concentrations of specific mRNAs in different cells?

MATERIALS AND METHODS

mRNA Isolation

mRNA was isolated from Chinese hamster liver by a modification of the technique described by Ullrich et al. (7). The liver was removed after cervical fracture from two-month old male Chinese hamsters, rinsed quickly in ice-cold PBS (phosphate buffered saline .15 M Na^+), and immediately homogenized in 6M guanidine thiocyanate (Fluka) in the presence of 100 mM Tris-HCl, pH 7.5 and 10% β-mercaptoethanol. The ratio of homogenizing solution to wet weight of tissue was about 10 ml per gram; larger volumes of solution resulted in loss of RNA while lower ratios were not sufficient to protect the isolated RNA against nucleolytic degradation. Homogenization was for 3 to 20 seconds in a 30-ml Waring blender. The homogenized suspension did not reach temperatures higher than 37°C under these conditions. Following homogenization, acetic acid was added to 50 mM followed by one volume of ethanol. Nucleic acid was precipitated for 60 minutes at -20°C, collected by centrifugation and redissolved in 7.5 M guanidine hydrochloride, with 100 mM Tris-HCl pH 7.5 and 1 mM dithiothreitol (5 ml/gram tissue). Acetic acid was added to 25 mM, followed by 0.5 volume of ethanol, the mixture was stored at -20°C for at least 60 minutes, the precipitate collected and this step was repeated twice. Guanidine hydrochloride was removed from the resulting pellet by washing with 95% ethanol at -20°C, followed by centrifugation. The washed pellet was dissolved in ETS (10 mM Tris-HCl pH 7.4; 10 mM Na_2 EDTA pH 7.4; 0.2% sodium dodecyl sulfate), 2 ml per gram initial weight. Deproteinization was accomplished by phenol: chloroform (1:1) extraction in the presence of 0.2% SDS (8); RNA was ethanol precipitated and re-extracted by ETS saturated butanol: chloroform, 4:1 (7); reprecipitated with 2 volumes of ethanol and resuspended in 3 M sodium acetate, pH 5.0 (500 µl/mg precipitate). RNA did not dissolve and was recovered by centrifugation and the sodium acetate wash was repeated twice. The final RNA precipitate was washed again with 95% ETOH and dissolved in ETS. Poly(A)-containing RNA was selected by poly(U)-Sepharose chromatography (8) which was repeated twice, to remove 18S rRNA contamination.

In vitro Iodination of mRNA

mRNA was iodinated and stored as previously described (10). In order to remove traces of iodinated proteins from the labeled probe, RNA was treated with proteinase K following the iodination procedure and then it was phenol extracted and ethanol precipitated.

DNA-excess Hybridization Conditions

Isolations of recombinant plasmid DNA were conducted in a P3 biocontainment facility as specified by the NIH Guidelines for Research Involving Recombinant DNA Molecules. Nine cDNA plasmids were used, the identification and properties of which

were previously described (6). The RNA·DNA hybridizations were carried out as described (6). All hybridizations were performed in the presence of 30 μg/ml HeLa cell rRNA, to compete with the 18S contaminant in the iodinated probe.

Labeling Conditions for Liver Cells

Chinese hamster livers were dissected, rinsed in PBS at 37°C and minced thorougly. Minced tissue was gently homogenized in a Thomas glass homogenizer at 4°C, three strokes in an A pestle, in 5 ml/gram tissue of warm Eagle's medium (11). Cells were spun 3 minutes at 1500 rpm and suspended in fresh medium at 4°C. Clumps of tissue were allowed to settle for 1 minute and the cell suspension decanted carefully. About 1 x 10^8 cells were obtained under these conditions from 1 gram of liver. Cells were counted and brought to a concentration of 1 x 10^7 cells/ml in MEM. The cell suspension was stirred with a magnetic bar for 10 minutes at 37°C prior to the addition of labeled uridine. ^{3}H-uridine (38-30 Ci/mMole) was added at a concentration of 0.25 mCi/ml (9 μM uridine) for 10 minutes. Cells were chilled with frozen medium, centrifuged 3 minutes at 1500 rpm and washed in ice-cold PBS. Total cellular RNA was prepared as described for the intact liver, using 5 ml of guanidine thiocyanate solution for 1 x 10^8 cells. About 1 x 10^7 cpm of ^{3}H-uridine were incorporated under these conditions into total cell RNA.

RESULTS AND DISCUSSION

To compare the concentration at steady state of the mRNA in liver cells with the previous results obtained with cultured Chinese hamster ovary cells (6), liver cell mRNA {poly(A)-containing RNA} was extracted, purified by poly(U)-Sepharose chromatography and labeled with I^{125}. A reasonable yield of mRNA per gram of liver tissue was obtained, ∿10 to 20 μgm/gm of tissue, which is roughly one-third to one-half of what is obtained from cultured cells. Prior to iodination, purified liver mRNA had a sedimentation profile similar to cultivated cells with a slightly smaller average size.

The fraction of the labeled input mRNA which formed hybrids with DNA from each of the nine recombinant plasmids was measured. In order to assure that sufficient DNA was used to hybridize all the labeled RNA, the supernatant fluid plus the RNA that could be washed from the first set of filters was hybridized against a second set of filters. Only 10 to 15% of the total complementary mRNA hybridized to the second set of filters. Several hybri-

TABLE 1

Hybridization of ^{125}I-Labeled Liver Cell mRNA

Clone	Liver CPM Hybridized Exp. Number 1	2	3	Liver Average % of Total mRNA in Hybrid[1]	CHO Average % of Total mRNA in Hybrid[2]	Ratio, Liver/CHO
A	2,716	2,907	2,606	.035	.055	0.64
B	5,299	15,990	5,831	.120	.22	0.54
C	2,335	4,638	2,349	.038	.030	1.3
D	2,296	2,701	2,283	.030	.012	2.4
E	1,685	1,335	1,518	.014	.0165	0.85
F	1,882	2,220	1,813	.023	.063	0.38
G	2,180	3,041	2,384	.031	.023	1.35
H	3,056	2,870	3,515	.042	.027	1.5
I	2,389	1,753	2,213	.029	.0021	13.7
PBR 322	493	888	524			
CPM input	5.3×10^6	1×10^7	5.4×10^6			

[1] The percent of mRNA hybridized to each cloned DNA fragment (CPM in hybrid/input CPM x 100) was calculated after subtracting the background shown for PBR-322 in each experiment and averaging the three experiments.

[2] The percent of labeled mRNA that was specific for each clone was determined in RNA from cells labeled with ^{3}H-uridine for 36 to 48 hours in five separate experiments (6; Harpold, Wilson and Darnell, unpublished data). The average of the five experiments is given here.

dization experiments were performed and the data is given in Table 1. In addition, the "hybridization background", labeled RNA bound to nitrocellulose filters containing plasmid DNA with no CHO sequences is also shown. The range of specific hybridization as assayed by pancreatic RNase resistant hybrids was from two to well over ten times background. Most important, the percentage of the input labeled RNA complementary to each of the nine clones was quite similar comparing CHO culture cells with liver for 8 of the 9 specific sequences. One of the nine, clone I, which on a molar basis in CHO cells is one of the scarcest mRNAs in the set of nine (6) was 13-fold higher in steady state concentration in liver cells than in CHO cells.

TABLE 2

Hybridization of Nuclear RNA to Specific Cloned CHO DNA Segments

	Liver			CHO			
Clone	CPM in Hybrid 1	CPM in Hybrid 2	% Input Hybridized	CPM in Hybrid 1	CPM in Hybrid 2	% Input Hybridized	Liver/CHO
A	281	366	.0035	1200	1050	.0082	.44-.88
B	241	278	.0029	632	950	.0063	.46-.92
C	185	328	.0029	506	360	.0030	.97-1.9
D	83	196	.0015	150	50	.0062	2.4-4.8
E	53	31	.00047	483	300	.0027	.17-.34
F	78	194	.0015	235	120	.0012	1.2-2.4
G	80	53	.00075	127	78	.00070	1.1-2.2
H	46	130	.00099	86	114	.00073	1.35-2.7
I	153	141	.00165	138	141	.0011	1.5-3.0
Input	10^7	7.8×10^6		4.0×10^7	2.0×10^7		

Chinese hamster liver cell nuclei were labeled for 10 minutes as described in Materials and Methods and total cell RNA prepared for hybridization. Earlier experiments with rodent liver cell incorporation indicates >90% of the labeled RNA after such a short label is nuclear (12). The CHO cells were labeled for 10 minutes and nuclear RNA prepared as described (6). The background on filters containing PBR 322 DNA with no CHO DNA were from 10 to 25 CPM and the numbers in the table are corrected for that background. One-half of the input radioactivity of the CHO cell RNA was assumed to be pre-rRNA in calculating the percentage of CHO cell nuclear RNA hybridizing to each clone (6). The percentage of input hybridized for the liver cell RNA is calculated with no correction; the ratio of liver/CHO is given with no correction of pre-rRNA (the lower number) or with 50% of the labeled liver RNA as pre-rRNA (the higher number).

To estimate the relative rate of synthesis in liver cells of RNA complementary to each of the nine specific mRNA sequences liver cell suspensions were exposed to ^{3}H-uridine for 10 min. The ^{3}H-uridine labeled nuclear RNA was extracted and hybridized to each of the nine cloned segments and the RNAse resistant hybridized RNA was measured. Again the percentage of the total pulse-labeled nuclear RNA which was complementary to each clone was compared and found similar to that found in the CHO nuclear RNA (Table 2). In cultured CHO cells, about one-half of pulse-labeled

RNA is in pre-rRNA and one-half is in hnRNA; the CHO data are therefore corrected to reflect that fact, i.e. the input of radioactivity is divided by two before calculating the percentage of hnRNA hybridized to a specific clone. However, the fraction of the total RNA synthesis in liver cells that is pre-rRNA is probably much less than in growing cells (12) but we do not know exactly what this fraction is. Therefore we have entered two numbers in Table 2 for the percentage of nuclear RNA hybridized to each clone, one assuming no rRNA synthesis in the liver and one based on 50% of the total pulse-label as pre-rRNA. The actual correction should doubtless be somewhere between the two.

With this relative indeterminancy in mind, it appears that the increase in the steady-state level of mRNA complementary to clone I (average of 13-fold increase in three experiments, Table 1) is greater than the more modest 1.5 to 3-fold increase in pulse-labeled nuclear RNA complementary to clone I. For example, newly synthesized nuclear RNA complementary to two other clones (D&F) showed approximately a 2-fold increase in liver compared to cultured cells but either a decrease or a more modest increase in steady-state cytoplasmic RNA. It appears possible from this data that sequences complementary to clone I might undergo both a modest increase in rate of synthesis and a somewhat greater degree of cytoplasmic stabilization in order to account for the 13-fold increase. In a similar finding, Guyette, Matusik and Rosen (13) have recently reported that the increase in casein mRNA in breast tissue in response to prolactin is accomplished by a modest (2 to 4-fold) increase in transcription but a 15 to 30-fold increase in mRNA stability.

The present results support the earlier conclusions (1-5) that many specific mRNAs will likely prove to be found in similar

concentrations in many (or most) cells of an animal. In these experiments we detected a substantial change (greater than 10-fold) in the concentration of one out of nine mRNAs. Whether this is an accurate guide to the frequency of such changes in various differentiated cells will of course require much more data.

It is true however that the new recombinant DNA technology will allow changes in mRNA concentration to be accurately measured so that molecular basis for these changes can be elucidated.

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