Frequency distribution of mRNA and pre-mRNA in growing and differentiated Friend cells

MAURON, Alex, SPOHR, Georges

Abstract

The frequency distribution of poly(A)+-mRNA in growing and in differentiated Friend cells has been measured by mRNA-cDNA hybridization and their differences established by heterologous hybridization of mRNA of one type and cDNA of the other. It was shown that induction of Friend cells involves an increase in abundance of a small number of mRNAs, while no specific pattern of messenger disappearance could be detected. The frequency distribution of pre-mRNA was determined by hybridizing nuclear RNA with the cDNA probes complementary to mRNA. In uninduced Friend cells, it was shown that most precursor messenger sequences are present at a single frequency of about 3 molecules per nucleus, independently of their final frequency in polysomal mRNA. In induced Friend cells, the frequency distribution of pre-mRNA is more heterogeneous and correlated to some extent with the corresponding mRNA frequency distribution.

Reference


PMID : 278967

Available at:
http://archive-ouverte.unige.ch/unige:85373

Disclaimer: layout of this document may differ from the published version.
Frequency distribution of mRNA and pre-mRNA in growing and differentiated Friend cells

Alex Mauron and Georges Spohr

Swiss Institute for Experimental Cancer Research, Ch. des Boveresses, 1066 Epalinges, Switzerland

Received 2 May 1978

ABSTRACT

The frequency distribution of poly(A)$^+$-mRNA in growing and in differentiated Friend cells has been measured by mRNA-cDNA hybridization and their differences established by heterologous hybridization of mRNA of one type and cDNA of the other. It was shown that induction of Friend cells involves an increase in abundance of a small number of mRNAs, while no specific pattern of messenger disappearance could be detected. The frequency distribution of pre-mRNA was determined by hybridizing nuclear RNA with the cDNA probes complementary to mRNA. In uninduced Friend cells, it was shown that most precursor messenger sequences are present at a single frequency of about 3 molecules per nucleus, independently of their final frequency in polysomal mRNA. In induced Friend cells, the frequency distribution of pre-mRNA is more heterogeneous and correlated to some extent with the corresponding mRNA frequency distribution.

INTRODUCTION

The control of gene expression in eukaryotic cells still defies a general explanation, partly because of the multiplicity of levels at which molecular regulation devices may operate. In order to appreciate the importance of these various levels of control, it would be important to know which sets of genes are transcribed into nuclear RNA and into messenger RNA in a given cell type.

Many studies have recently been performed in which the diversity of the sequences in mRNA and in nuclear RNA of different cells has been measured. The results of such measurements are expressed in terms of the sequence complexity of an RNA population, i.e., the total number of RNA species present. For instance, the sequence complexity of mRNA from sea urchin gastrula has been determined by hybridizing radioactive non-repetitive DNA with an excess of mRNA (1). At saturation, the proportion of DNA taken into hybrid form is a measure of the complexity of mRNA. On the other hand, the kinetics of this hybridization is also related to the complexity. From both these
data taken together, it was possible to conclude that different mRNA species occur at widely different frequencies. Another technique for determining sequence complexity is best suited to study the frequency distribution of mRNA populations: polyadenylated mRNA is used as a template in the synthesis of a cDNA probe which is back-hybridized with its template. The kinetics of this RNA-excess hybridization is related to the sequence complexity of the mRNA. It usually shows a pattern of multiple transitions which suggests that the frequencies of individual messenger species are clustered around 2 to 4 discrete values, rather than taking any value in a broad, continuous range. In other words, the quantitative structure of an mRNA population could be approximately described by dividing it into 2 to 4 abundance classes (2-4).

In cell differentiation, control mechanisms are thought to operate in order to express the genes necessary for the performance of specialized cell functions. Thus, the sets of sequences found in mRNAs of various cell types presumably differ to some extent. The hybridization techniques just described allow us to observe the effects of cell differentiation at the mRNA level. Comparisons between the mRNA populations of different cell types of various origins have been made by heterologous mRNA-non-repetitive-DNA or mRNA-cDNA hybridization (5-13). A more or less extensive overlap between the mRNA of different tissues is usually found, with some messenger sequences being specific for a certain cell type. In addition, sequences common to different cell types may differ in abundance, again stressing the importance of the quantitative, as well as the qualitative composition of mRNA populations.

The sequence complexity of nuclear RNA has also been measured (sea urchin (14), drosophila cells (15), rat tissues (16), mouse brain (17), uninduced Friend cells (18, 19). It has usually been found to be 5-10 times higher than the complexity of polysomal mRNA.

We have chosen to study the frequency distribution of mRNA and of pre-mRNA of Friend cells, an established line of mouse erythroleukemic cells transformed by an RNA tumor virus (20). These cells resemble the proerythroblast stage of normal red blood cell differentiation but upon addition of a variety of inducing agents, in particular dimethylsulfoxide (DMSO), they experience a differentiation process whose biochemical parameters have been extensively characterized. In the presence of the indu-
Nucleic Acids Research

cing agent, there is an accumulation of many proteins specific for terminal-
ly differentiated erythrocytes, such as α and β globin (21), spectrin
(22), acetylcholinesterase (23), enzymes involved in the biosynthesis of
heme (24), etc. In the case of uninduced Friend cells, the sequence com-
plexity of polyadenylated mRNA had already been determined by Birnie et
al. (3). These authors showed that the mRNA contains about 10⁴ different
sequences divided into 3 abundance classes. Our first aim was to measure
the sequence distribution of polyadenylated mRNA before and after diffe-
rentiation and to determine the nature and extent of the differences be-
 tween the two stages. This was achieved by synthesizing the cDNA probes
complementary to the poly(A)⁺-mRNA of both stages and performing the homo-
logous and heterologous hybridizations with both mRNAs.

In addition, we wanted to gain some insight into the origin of the
quantitative structure of mRNA populations, i.e. their distribution in
discrete frequency classes. We asked the question whether the subclass of
nuclear RNA containing the messenger sequences to be expressed as polysom-
al mRNA is organized in the same quantitative structure as polysomal
mRNA. In other words, we wanted to know whether a sequence which is abun-
dant in polysomal mRNA is already abundant in this subclass (pre-mRNA).
We had observed that in Friend cell line F4N, according to the differen-
tiation stage, there is a correlation between the concentration of the
globin sequence in nuclear RNA and the abundance of globin mRNA in the
cytoplasm (this is different in other cell lines). In order to determine
whether this is a general property of polyadenylated mRNAs, we hybridized
total nuclear RNA of both types of Friend cells with the cDNA probes com-
plementary to mRNA. From the kinetics of hybridization, we could establish
the frequency distribution of pre-mRNA in growing and in differentiated
Friend cells.

MATERIALS AND METHODS

1. Cell culture

Friend cells (line F4N isolated by Ostertag and obtained from Dr. H.
Eisen) were grown in suspension in Dulbecco’s modified Eagle’s medium sup-
pplemented with 15% fetal calf serum (Gibco) and a doubled concentration of
glutamin. The cells were induced by adding 1,25% DMSO and diluting every
day with DMSO-containing medium.

3015
Nucleic Acids Research

2. Cell fractionation

All steps were carried out at 4°C. Cells were collected by centrifugation at 280 g. The pellet was resuspended in 10 vol. of Earle’s saline and recentrifuged. Washed cells were taken up in 8 vol. of lysis buffer (10 mM Triethanolamine-HCl pH 7.4, 10 mM NaCl, 1 mM MgCl₂, 1 mM MnCl₂). After 3 min., isotonicity was restored by adding sucrose (0.25 M). 0.1% Triton-X-100 was added and the cells lysed with a dounce homogenizer. The lysate was layered on a 10 ml. cushion containing 1M sucrose in lysis buffer adjusted to pH 6.9 and centrifuged for 10 min. at 630 g. The upper layer containing the cytoplasmic material was centrifuged for 20 min. at 10000 g. and the supernatant further processed to obtain polysomes. The interface was discarded and the nuclear pellet was resuspended in 0.25 M sucrose pH 6.9 lysis buffer containing 0.1% Triton-X-100. Nuclei were centrifuged for 5 min. at 630 g., the pellet resuspended as before and kept over liquid nitrogen until further processed.

3. Preparation of Friend cell mRNA

The cytoplasmic material was layered on a 10 ml. cushion containing 15% sucrose in lysis buffer pH 7.4 and centrifuged in a fixed angle rotor (Beckman Ti 60) for 60 min. at 60000 RPM and 4°C. The polysomal pellet was resuspended in 0.25 M sucrose in lysis buffer pH 7.4 and extracted with phenol-chloroform according to Perry et al. (25). Total polysomal RNA was subjected to oligo (dT)-cellulose chromatography (26). The RNA was redissolved in high-salt buffer (10 mM Tris-HCl pH 7.5, 0.5 M NaCl, 1 mM EDTA, 0.2% SDS) and passed three times over an oligo(dT)-cellulose column at room temperature. After rinsing with 3 column volumes of buffer, the polyadenylated RNA was recovered by elution with prewarmed low-salt buffer (without NaCl). After heat denaturation of the eluate (2 min. at 100°C), the chromatography was repeated and the second poly(A)+ pool was used in cDNA synthesis and hybridizations.

4. Preparation of Friend cell nuclear RNA

The nuclear fraction (see section 2) was extracted with hot phenol as described by Scherrer (27). The recovered aqueous phase was precipitated twice with ethanol and treated with DNase (10 μg/ml RNase-free grade, Worthington) in the presence of 2 mM MnCl₂. The digestion (20 min. at 4°C) was stopped by addition of EDTA (10 mM), SDS (0.2%), and adjusted to 0.15 M NaCl. The RNA was then subjected to exclusion chromatography on Bio-Gel
A5m (Bio-Rad) equilibrated with 20 mM Tris-HCl pH 7.5, 0.15 M NaCl, 0.2% SDS, 1 mM EDTA, 5 U-USP/ml heparin.

5. Preparation of globin mRNA

Globin mRNA was prepared from anemic mice as described by Williamson (28) using phenol-chloroform extraction and oligo(dT) cellulose chromatography.

6. Synthesis of cDNA

cDNA was synthesized with reverse transcriptase as described by Imaizumi et al. (29).

7. Hybridization

RNA-DNA hybridizations were performed as described by Imaizumi et al. (29) with slight modifications. The hybridization mixture contained the RNA sample, 0.3 M NaCl, 50 mM Tris-HCl pH 7.5, 0.1 mg/ml of E.Coli 4S RNA, 0.1% SDS and 200-300 cpm of cDNA. The mixture was sealed in microcapillary pipettes in portions of 10 μl, incubated at 65°C for various times and flushed into 100 μl of S1 digestion mixture (30 mM sodium acetate pH 4.5, 25 mM NaCl, 1 mM ZnSO₄, 6% glycerol, 6 μg/ml of heat-denatured salmon sperm DNA and 5 units/ml of S1 nuclease prepared according to Vogt (30)). The incubation was carried out at 45°C for 40 min. The proportion of cDNA taken in hybrid form was calculated using undigested standards and subtracting the zero-time value at the lowest RNA concentrate from every time-point (5-7% of total).

8. Data reduction

An RNA-cDNA hybridization in RNA excess follows first-order kinetics, thus the proportion of DNA taken into hybrid form as a function of \( R_0 t \) is given by:

\[
H = 1 - e^{-k R_0 t}
\]

where \( R_0 \) is the concentration of RNA in mole of nucleotide per liter and \( t \) the hybridization time in seconds. The rate constant \( k \) is related to the sequence complexity of RNA and can be computed from the kinetic curve by taking the \( R_0 t \) value corresponding to \( H = 0.5 : R_0 t_{1/2} = \ln 2/k \).

If the RNA consists of various sequences clustered into \( N \) frequency classes, i.e. if the frequency of every mRNA type is one of \( N \) values, it is easy to show that eq. 1 becomes:

\[
H = 1 - \sum_{n=1}^{N} p_n e^{-k n R_0 t}
\]

(Bishop et al., 2)
where \( p_n \) is the relative mass of RNA comprising the mRNA types of the \( n \)-th frequency class and \( k_n \) the corresponding rate constant. We assume that the same values \( p_n \) apply to the cDNA. This assumption is sound, as shown by Hastie and Bishop (7). The fitting of eq. (2) to the experimental data was performed using a non-linear least squares routine on a CDC CYBER 7326 system. Having been provided with the data, the mathematical model and a choice of \( N \), this program computes the values of \( p_n \) and \( k_n \) giving the best fit and the relevant standard errors and confidence intervals.

**RESULTS**

**Frequency distribution of uninduced cell poly(A)\(^+\)-mRNA**

We measured the sequence complexity of poly(A)\(^+\)-mRNA of uninduced and fully induced (4 days in \( \sim 0.25\% \) DMSO) Friend cells. Total polyribosomal poly(A)\(^+\)-mRNA from uninduced cells was transcribed into \(^3\)H-labeled cDNA (specific activity about \( 10^4 \) cpm/ng). Fig. 1 shows the hybridization curve of mRNA from uninduced cells with the cDNA transcribed from it: an excess of mRNA was incubated with cDNA for various time-periods and the fraction of cDNA in hybrid form determined as the proportion of DNA resistant to
the single-stranded specific nuclease S1. In order to make a comparison on
the same graph with a hybridization curve obtained with a single kinetic
component we show on the same figure the hybridization kinetics of total
polysomal RNA (not purified poly(A)$^+$-mRNA) of uninduced cells with globin
cDNA. The abscissa of the latter curve has been shifted by a factor of 50,
expressing the fact that poly(A)$^+$-mRNA represents approximately 1/50 of
total polysomal RNA. The curve obtained by hybridizing uninduced cell mRNA
with its cDNA shows two transitions, suggesting two abundance classes.
Therefore, the data have been reduced to a model involving two kinetic
components using the non-linear least squares technique. The relevant nume-
rical data are summarized in Table 1. In determining the fraction of each
abundance class, the assumption was made that the cDNA remaining unhybri-
dized at very high $R_t$ values does not belong to any particular abundance
class. The rate constant for each class provided by the computer calcula-
tion was converted to $R_{0.5}$ using the relation: $R_{0.5} = \ln 2/k$. The value
of $R_{0.5}$ is proportional to the sequence complexity of the mRNA in a given
kinetic component. The standard of complexity is provided by the hybridiza-
tion of mouse globin mRNA with its cDNA. Reticulocyte 9S globin mRNA is
a mixture in equal amounts of the mRNAs coding for the $\alpha$- and $\beta$-chain.
The summed MW of these two messengers is $4 \times 10^5$ dalton and we obtain a
$R_{0.5}$ value of $4 \times 10^{-4}$ mole $\text{mole}^{-1}$ in our hybridization conditions. Conside-
ring that an mRNA of number average size has a molecular weight of 600,000
dalton, the standard $R_{0.5}$ value, i.e. the $R_{0.5}$ corresponding to a comple-
xity of 1 average mRNA, will be

$$R_{0.5} = \frac{600,000 \times 4 \times 10^{-4}}{400,000} = 6 \times 10^{-4} \text{ mole m ole}^{-1}$$

Our results show that poly(A)$^+$-mRNA can be ordered into two frequency
classes. The first, low complexity class contains about 160 frequent types
of messengers. The other contains the rare messengers, which make up most
of the sequence diversity of mRNA which corresponds to $10^4$ different spe-
cies. As shown by Fig. 1 and by Table 1, in uninduced cells the globin
mRNA is a quite typical member of the latter class.

In order to determine the frequency distribution of mRNA in induced
Friend cells, cDNA transcribed from poly(A)$^+$-mRNA of induced cells was hy-
bridized to its own template. As shown in Fig. 2, the hybridization takes
<table>
<thead>
<tr>
<th>Abundance class no.</th>
<th>A. Fraction of cDNA in hybrid</th>
<th>Fraction of cDNA in frequency class</th>
<th>$R_{0.5}$ of pure component</th>
<th>Sequence complexity (number of different mRNAs of average size)</th>
<th>B. $R_{0.5}$</th>
<th>$2^\text{proportion of globin sequence in total poly(A)$^+$$-mRNA}$</th>
<th>$3^\text{Number of globin mRNA molecules per cell}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.414</td>
<td>0.44</td>
<td>0.098</td>
<td>163</td>
<td>371</td>
<td>5.4 x 10^{-5}</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>0.536</td>
<td>0.56</td>
<td>5.9</td>
<td>9870</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numerical data from the hybridization curves in Fig. 1. A. Hybridization of uninduced cell poly(A)$^+$$-mRNA with its own cDNA.

1. The number of molecules of each type is calculated in the following way: uninduced Friend cells contain about 30 pg/cell of total RNA, which we take as a reasonable estimate of polysomal RNA. We estimate 2% of this amount to be poly(A)$^+$$-mRNA, i.e. 30 pg x 0.02 = 0.6 pg or 0.6 x 10^{-12} g. Since the number average molecular weight of these molecules is 6.8 x 10^2 g/mole, their total number will be equal to (0.6 x 10^{-12} g x 6 x 10^{23} mole^{-1}) / 6 x 10^2 g/mole = 600,000 where 6 x 10^{23} is Avogadro's number. Of these 600,000 molecules, 264,000 belong to the first abundance class and 336,000 to the second. Dividing these figures by the corresponding complexities, one obtains the number of molecules of each sequence. B. Hybridization of total uninduced cell polyribosomal RNA with globin cDNA.

2. The proportion of globin sequence in total polysomal RNA is given by the ratio of $R_{0.5}$ for the globin mRNA-globin cDNA hybridization to the $R_{0.5}$ observed with polysomal RNA: $4 x 10^{-5} / 371 = 10^{-6}$. Since poly(A)$^+$$-mRNA is 2% of polysomal RNA the corresponding fraction of globin sequence is $10^{-6} / 0.02 = 5.4 x 10^{-5}$. In the same way as in A, the number of globin mRNA molecules is given by $0.6 x 10^{-12} g x 5.4 x 10^{-5} x 6 x 10^{23} mole^{-1}) / 4 x 10^2$ g/mole = 49 introducing the molecular weight of the $\gamma$+ $\beta$ globin mRNAs instead of the number average molecular weight of all mRNAs.
Complexity of induced cell mRNA. Poly(A)^+ mRNA was purified from induced cells and hybridized with its own cDNA at mRNA concentrations of 30 to 180 µg/ml. The broad transition in the higher R_Q range comprises two first-order components which could not be accurately resolved. This has little effect on the parameters for the most abundant class which covers 23-27% of the hybridization and has a complexity of 4-6 messenger sequences, whatever assumptions are made for the middle and high-complexity class. Since induced cells contain 15 pg of RNA, this corresponds to 13000-19000 molecules per species per cell. Hybridization with globin cDNA is also shown and the same comment as in Fig. 1 applies. (e — e) Hybridization of induced cell mRNA with the corresponding cDNA. (o — o) Hybridization of induced cell polysomal RNA with globin cDNA.

place over a larger range of R_Q values than in the uninduced situation. Computer analysis of the data shows that, contrary to the previous case, the fit of these data to a 2-component model is not satisfactory. In addition, significant hybridization takes place at lower R_Q values than in Fig. 1, meaning that a few mRNA species must be present at a very high frequency. This is confirmed by the calculation, which shows that a three-component model is appropriate. The first of the three kinetic components, comprising 23-27% of the messenger mass corresponds to only 4-6 different sequences. These sequences are present at 13'000 to 19'000 molecules/cell. The middle and high complexity class could not be resolved in a reliable way but comparing the data to those in Fig. 1 shows that they are not grossly different in the higher R_Q range. Thus, the total complexity does not seem to change significantly from the uninduced to the induced stage (this is further confirmed by the heterologous hybridizations described later). As in the previous experiment, induced-cell total polysomal RNA has been hybridized with globin cDNA and the same remarks apply for the scale-shift. The
result shows that the globin mRNA is one of the 4-6 mRNAs in the highest frequency class. (Table 2)

Having established the quantitative structure of the mRNA population in both types of cells, we wished to establish the differences between them. Messenger species present in one state could be totally absent in the other; furthermore, quantitative rearrangements could take place during induction such as frequent mRNAs becoming rarer or vice-versa.

In order to investigate these possibilities, we have done a heterologous hybridization: mRNA from induced cells has been hybridized with cDNA complementary to uninduced cell mRNA. Fig. 3 shows that the final level of hybridization (90%) is not significantly different from that of the homologous reaction involving the same cDNA (Fig. 1). Moreover, the shape of both curves is indistinguishable but slightly shifted to higher Rot in the heterologous reaction. This is confirmed by the computer curve fitting which yields similar proportions and similar relative rate constants for both classes. (The uncorrected proportion of the abundant class in the homologous reaction is $0.414 \pm 0.016$ (standard error), as shown in

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>A. Fraction of cDNA in 1st abundance class</th>
<th>$R_{0}$ $%$ of pure component</th>
<th>Sequence complexity (number of different mRNAs of average size)</th>
<th>Number of molecules of each mRNA per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.23 - 0.27</td>
<td>0.0022-0.0038</td>
<td>3.6 - 6.3</td>
<td>13000-19000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B.</th>
<th>$R_{0}$</th>
<th>Proportion of globin sequence in total poly(A)$^+$-mRNA</th>
<th>Number of globin molecules per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.79</td>
<td>0.025</td>
<td>11300</td>
</tr>
</tbody>
</table>

Numerical data from the hybridization in Fig. 2. A. Hybridization of induced cell poly(A)$^+$-mRNA with its own cDNA. Close inspection of the computer fitting reveals that the parameters pertaining to the 2nd and 3rd abundance class are not uniquely defined by the data (in other words, these two kinetic components are not well separated). But it also shows that this uncertainty barely affects the parameters for the 1st class, which are comprised in the range indicated. The calculations are similar to the ones in Table 1, taking 15 pg as the amount of RNA per induced cell. B. Hybridization of total induced cell polysomal RNA with globin cDNA.
Heterologous hybridization: induced cell mRNA with uninduced cell cDNA. Poly(A)^+-mRNA obtained from induced cells was hybridized with cDNA transcribed from uninduced cell mRNA. The data were fitted to a two component model. For the purpose of comparison, the curve fitting of the homologous reaction is also shown. (••) Heterologous reaction: data points and curve fitting. (----): uninduced cell mRNA with uninduced cell cDNA, curve fitting from Fig. 1.

Table 1 while the corresponding value in the heterologous reaction is 0.448 ± 0.013). This result is consistent with the interpretation that, except for the 4 to 6 mRNAs which are greatly increased, the vast majority of the messenger RNAs which are present in the uninduced state are still expressed in the induced cell and present at the same relative frequency. Moreover, since the heterologous hybridization is essentially complete, there must be little or no qualitative differences between the two populations of messengers.

This point was confirmed by performing the opposite heterologous hybridization: mRNA from uninduced cells has been hybridized with cDNA complementary to induced cell mRNA. In this experiment, we probe the mRNA population of uninduced cells for the existence and frequency of sequences characteristic of the differentiated state. As shown in Fig. 4 this heterologous reaction also goes to completion. As compared to the homologous reaction in the induced case shown in the same figure, this heterologous hybridization does not show the fast-reacting component. Therefore, most messengers abundant in the induced cell are already present in the uninduced stage, but they hybridize at higher R_0t, which means that they are present at a lower frequency.

In summary, it appears that at the level of the population of mRNA
sequences, the differentiation of Friend cells involves mainly an increase in abundance of a small subset of messenger sequences already present before induction. As a result of this increase, a few mRNA species reach concentrations which are not found for any messenger type in the undifferentiated cells. On the other hand, based on the heterologous hybridization in Fig. 3, we are unable to detect any specific pattern of messenger disappearance linked to cell differentiation.

**Quantitative distribution of messenger sequences in pre-mRNA of uninduced cells.**

The sequence complexity of total poly(A)$^+$-nuclear RNA of uninduced Friend cells had been measured using a cDNA probe transcribed from it (Getz et al. 18). It has been found to be 5 times higher than the complexity of poly(A)$^+$-mRNA, indicating that only a fraction of poly(A)$^+$-nuclear RNA is pre-mRNA. Furthermore, the population of polyadenylated nuclear RNA can be ordered into 2 frequency classes. Our aim is to know whether at the level of nuclear RNA, the messenger sequences are distributed with the same relative frequencies as in mRNA itself. In other words, we shall consider the frequency distribution not of hnRNA as a whole, but of the subset of hnRNA sequences which consist of messenger sequences (pre-mRNA). It is important to realize that the result of Getz et al. on total poly(A)$^+$
nuclear RNA does not allow one to draw conclusions about the frequency distribution of pre-mRNA: pre-mRNA is only a subset of total poly(A)$^+\text{nuclear RNA, comprising 20% of its complexity. Therefore, if pre-mRNA consisted of multiple frequency classes, they might not be resolved in a hybridization involving the whole of polyadenylated nuclear RNA.}

Total nuclear RNA of uninduced cells has been hybridized with cDNA complementary to mRNA of uninduced cells. If the relative abundance of messenger sequences in mRNA and pre-mRNA do correspond, the $R_{\theta\chi}$ curve should be parallel to the curve in Fig. 1 and shifted to the right by a factor expressing the dilution of the reacting messenger sequences by unrelated sequences. The result (Fig. 5) shows that this is not the case. Rather than being biphasic, the curve looks parallel to the single-component hybridization curves involving globin cDNA. Therefore, within hnRNA, most of the messenger sequences hybridize as a single abundance class whose $R_{\theta\chi}$ equals 690. Since the complexity of mRNA is about $10^9$ average messenger species and the corresponding $R_{\theta\chi}$ equal to 6 mole $x$ mole$^{-1}$ (Table 1), the proportion of nuclear RNA driving the reaction, i.e. the relative mass of the pre-messenger sequences in total nuclear RNA is 6/690 = 0.87%. The amount of total nuclear RNA has been determined by measuring the RNA and DNA content of nuclear preparations with orcinol and diphenylamine respectively (31) and found to be about 3 pg/nucleus. This corresponds to $0.0087 \times 3 \times 10^{-12} \text{g} \times 6 \times 10^{23} \text{mole}^{-1} / 6.10^5 \text{g/mole} = 26100$ messenger-sized sequences or 26100/10000 = 3 molecules of each species approximately. The result shows that about 3 molecules of every messenger sequence expressed in the uninduced cell are present in its nucleus, which agrees with the abundance of the complex class of total poly(A)$^+$-hnRNA found by Getz et al. (6 molecules/nucleus).

Quantitative distribution of messenger sequences in pre-mRNA of induced cells

In order to see whether in induced cells, a similar difference between the frequency distribution of mRNA and pre-mRNA can be observed, induced cell nuclear RNA has been hybridized with cDNA transcribed from induced cell poly(A)$^+$-mRNA. Relative to the uninduced cell situation, the hybridization curve is shifted toward the lower $R_{\theta\chi}$ values, indicating a relative enrichment in precursor messenger sequences. In addition, the curve in Fig. 6 extends over a slightly broader range of $R_{\theta\chi}$ values, indi-
Frequency of messenger sequences in nuclear RNA from uninduced cells. Total nuclear RNA prepared from uninduced cells was hybridized to cDNA complementary to uninduced cell poly(A)+-mRNA. The RNA concentration was 4.4 mg/ml. A similar hybridization has been performed with globin cDNA. (• - •) Hybridization of uninduced cell nuclear RNA with cDNA transcribed from uninduced cell mRNA. ( o ) Hybridization of uninduced cell nuclear RNA with globin cDNA.

Fig. 5
Frequency of messenger sequences in nuclear RNA from induced cells. Total nuclear RNA has been prepared from induced cells and hybridized to cDNA complementary to induced cell poly(A)+-mRNA at an RNA concentration of 3.3 mg/ml. A similar preparation has been hybridized with globin cDNA. (• - •) Hybridization of induced cell nuclear RNA with cDNA transcribed from induced cell mRNA. ( o - o) Hybridization of induced cell nuclear RNA with globin cDNA.

dicating kinetic heterogeneity. Therefore, the pre-mRNA of induced cells seems to be more heterogeneous in its frequency distribution, and precursor messenger sequences are enriched to varying extents as compared to the uninduced situation. As shown by hybridizing the same RNA with globin cDNA, this enrichment is higher for the globin sequence which is increased.
100-fold in induced nuclear RNA relative to uninduced nuclear RNA.

The possibility still exists that the globin sequence is an exception and that for most sequences the relative increase observed after induction is non-specific and due, for instance, to a decrease in precursor ribosomal RNA. Alternatively, this increase could be specific for the sequences enriched in induced-cell mRNA. This could be tested by a heterologous hybridization in which induced-cell nuclear RNA is reacted with the cDNA probe for uninduced mRNA. We have hybridized a single induced-cell nuclear RNA preparation with both cDNA probes, that is, we repeated the homologous reaction (induced cDNA) and made the heterologous reaction using the same material. Fig. 7 shows in dotted lines the two homologous reactions taken from Fig. 5 and 6.

The first set of data points is a repetition of the homologous reaction in the induced situation and therefore the points match the curve taken from Fig. 6. The second corresponds to the heterologous reaction of the induced-cell nuclear RNA with the cDNA complementary to uninduced-cell mRNA; this data set can be seen to match the homologous reaction curve for the uninduced case. Therefore, the higher frequency found for induced-cell pre-mRNA is not due to a general enrichment in pre-messenger sequences but is specific for those mRNAs which are more abundant in the cytoplasm after induction. Induced-cell pre-mRNA is more heterogeneous in its frequency distribution which shows some correlation with the abundances of the cytoplasmic mRNAs.

DISCUSSION

In these experiments, we have dealt with uninduced and induced cells as if they were homogeneous populations. This is very nearly so in the uninduced state, since one finds less than 0.1% of spontaneously differentiated cells. Cell heterogeneity may be more of a problem in the induced stage: due to the stochastic nature of the commitment to induction (32), no pure population of completely differentiated cells can ever be obtained. In the extreme, one could argue that the heterologous hybridization in Fig. 4 would be due to the undifferentiated cells contaminating the induced population. These cells represent not more than 10% of the total, but they contain twice as much polysomal RNA. This 20% contamination is insufficient to explain the data in Fig. 3 but it may influence them by making the
Fig. 7
Homologous and heterologous reaction involving induced-cell nuclear RNA. A single induced-cell nuclear RNA preparation was used in homologous and heterologous hybridization (cDNA transcribed from induced and from unin-
duced-cell mRNA respectively). The RNA concentration in both reaction
mixtures was 3.3 mg/ml. (• - •) Homologous reaction. (o - o) Heterologous
reaction. (---) Homologous reactions taken from Fig. 4 and 5.

heterologous curve more similar to the homologous one. Therefore, apart
from the very drastic increase of 4 to 6 mRNAs, other frequency changes
may occur and go undetected, either because of their low amplitude or
because they involve only a minority of the messenger species in a freque-
cy class. Furthermore, the fact that no specific pattern of mRNA
disappearance can be detected must also be considered in view of the
limited resolution of the hybridization technique. Such experiments do not
allow, in most cases, to detect changes in single mRNAs. However, Peterson
and McConkey have established protein patterns of uninduced and induced
Friend cells by two-dimensional electrophoresis: 98% of the 500 proteins
that they are able to resolve are similar in quantity and quality in both
types of cells and they observe the decrease or disappearance of only
four chromatin proteins and two cytoplasmic proteins. These findings
confirm that there is a high degree of similarity between the two mRNA
populations.

We have found that the mRNA of uninduced Friend cells is distributed
into 2 frequency classes. Similar experiments had already been performed
by Birnie et al. (3) leading to a similar estimate of the total complexity
but with a distribution of mRNA in 3 classes. In order to appreciate fully
the difference between their results and ours, one should bear in mind the
use of more kinetic components is bound to improve the fit between the data
and the model. The element of arbitrariness lies in the choice of a significance threshold and we consider a 2-component model to be satisfactory. Fortunately, the number of components affects very little the overall complexity found \((10^4\) messenger sequences).

Our experiments with nuclear RNA from uninduced cells show that pre-mRNA is organized into a single abundance class and corresponds approximately to 3 molecules per nucleus. Comparison of this result with the experiment by Getz et al. already shows that pre-mRNA sequences are not enriched over other poly(A)\(^+\)hnRNA sequences which do not correspond to messenger species found in the uninduced Friend cell. It also shows that the more abundant poly(A)\(^+\)-hnRNA sequences found by Getz et al. are apparently not precursors to the abundant mRNAs, at least not the majority of them. The same authors have measured the complexity of uninduced cell total nuclear RNA by hybridizing it with single-copy DNA \((19)\) : nuclear RNA transcribed from single-copy genes is four times more complex than mRNA and comprises \(3\%\) of total nuclear RNA. Interestingly, we find that pre-mRNA amounts to \(0.8\%\) of total nuclear RNA, i.e. about \(1/4\) of the complex class of nuclear RNA \((0.8/3 = 1/4)\) : the ratio of the amounts matches the ratio of the complexities, further supporting the view that pre-mRNA sequences are not enriched over other transcripts not to be exported into the cytoplasm.

In conclusions, our results with uninduced Friend cells suggest that posttranscriptional events are important on a quantitative level, since most prospective messenger sequences have the same abundance in the nucleus and are not more abundant than other transcripts from unique sequences.

The situation is somewhat different in the induced cells where pre-mRNA is enriched to varying extents in sequences found to be abundant in the cytoplasm. This could mean that for induced mRNAs, transcriptional activation is more important. On the other hand, this result may be due to contamination of our nuclear preparation by cytoplasmic material. Considering the concentration of the globin sequence in polysomal and in nuclear RNA of induced cells, this contamination should amount to at least \(10\%\) in order to explain our results. However, in uninduced cells such a level of cytoplasmic contamination would lead to a 15-fold enrichment of abundant messenger sequences over the amount found for total
sequences. Since this would certainly be detectable in Fig. 5, cytoplasmic contamination is not a likely explanation for our result with the nuclear globin sequence.

Contamination of polysomes by nuclear material would have an effect on our experiments since it would contribute RNA of high complexity to the polysomal fraction used to prepare the cDNA. However, the lysis procedure had been devised to purify polysomes which tested by centrifugation in a CsCl gradient, appear to be free of nuclear RNPs (Spohr et al., 34). Nevertheless, even if we allow for a contamination of 10% of poly(A)^+ mRNA by poly(A)^+ nuclear RNA, this would contribute a maximum of 10% of cDNA sequences of nuclear origin (probably less, since on a weight basis, poly(A)^+ nuclear RNA is reverse-transcribed less than poly(A)^+ mRNA). The complex component of polyadenylated nuclear RNA is about 5 times more complex than poly(A)^+ mRNA (18) and would therefore hybridize with its cDNA at a Rot of 30 in our conditions. In the experiment of Fig. 1, this contaminant of 10% would therefore hybridize at a Rot of 300 or more; it would be present as an additional minor complexity class, which is not observed, rather than merging into the complex class of mRNA.

An approach similar to ours was used by Sippel et al. (35) and Jacquet et al. (36) on rat liver and embryonal carcinoma cells respectively. In both studies, the frequency distribution of messenger sequences in nuclear RNA was found to be narrower than in mRNA. Kinetic studies involving the globin messenger sequence in Friend cells were also performed. Aviv et al. (37) have demonstrated differential turn-over of globin mRNA as compared to total poly(A)^+ mRNA while Orkin et al. (38) have shown an increase of the transcriptional rate of the globin gene during differentiation. Similarly, our results suggest that the generation of the quantitative structure of the mRNA population in a cell cannot be ascribed to a single mechanism. Post-transcriptional modulation probably does occur, while transcriptional activation might be most important for the few very predominant mRNAs which appear in terminally differentiated cells.

Acknowledgements

We thank Raymonde Cornuz for excellent technical assistance. We are deeply indebted to Bernhard Hirt for his continuous support and advice.
We acknowledge the gift of reverse-transcriptase by Heidi Diggelmann and Joseph Beard, The Office of Program Resources and Logistics, Viral Cancer Program, National Cancer Institute, Bethesda, MD. We thank Doris Chiapparelli, Sophie Cherpillod and Pierre Dubied for help in preparation of the manuscript and David Hughes for critically reading it. We also thank David Appleby and Yves Depeursinge for assistance in the computer work, which was performed at the Computer Center of the Ecole Polytechnique Fédérale de Lausanne. Supported by the Swiss National Science Foundation (grant No. 3.537.75)

* To whom requests for reprints should be sent at his present address: Département de Biologie Animale de l'Université de Genève, route de Malagnou 154, CH-1224 Chêne-Bougeries, Switzerland

References