Gene Expression and the Diversity of Polyosomal Messenger RNA Sequences in Regenerating Liver*

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We have determined the percentage of the genome transcribed into nuclear RNA and the sequence complexity and diversity of polyosomal mRNA in the livers of sham-operated and partially hepatectomized rats. Both in livers of sham-operated rats and in regenerating livers at the time of maximal DNA synthesis, approximately 12% of the single copy genome is expressed. The complexity of polyosomal polyadenylated RNA from livers of sham-operated and partially hepatectomized rats was determined by kinetic analysis of hybridization curves of polyosomal mRNA and [3H]eDNA. The sequence complexity of polyosomal polyadenylated RNA in 12-, 24-, and 72-h regenerating liver ranged from 10,000 to 20,000 sequences and did not differ from that of nongrowing liver, with the exception of the 72-h value. However, heterologous hybridizations between mRNA from regenerating livers and [3H]eDNA transcribed from polyosomal mRNA from livers of sham-operated rats as well as the converse reactions, did not provide evidence for the existence of polyosomal mRNA sequences which are specific for either regenerating or nongrowing livers. These studies suggest that changes in frequency of various sequences, rather than qualitative differences, constitute the major alteration in polyosomal mRNA populations during liver regeneration. The same conclusion applies to the regenerating liver of weanling rats which show a heightened and more synchronized regenerative response than adult rats.

Liver cells of adult rats rarely divide, but under appropriate stimuli, such as the removal of part of the liver mass, hepatocytes reacquire the ability to proliferate. At the peak of the regenerative response in rats, more hepatocytes undergo replication than in benign or malignant hepatic tumors (see Ref. 1 for review). In contrast to neoplastic growth which has a variable degree of autonomy, liver regeneration ends when the original mass of the organ is restored. This suggests that liver regeneration following partial hepatectomy is closely dependent on the animal's requirement for liver function. Growth control in this situation is independent of the gross morphology of the organ since the number, size, and shape of the liver lobes of the regenerated liver are quite different from that of the intact organ, despite the similarity of mass.

Because mRNAs exert a controlling role in most cellular functions, it is likely that alterations in mRNA populations are related to change in the growth capacity of hepatocytes. The long range goal of our work is to determine the extent to which mRNA populations in normal, regenerating, and neoplastic liver may differ quantitatively and qualitatively from each other, and to identify mRNA populations which might be specific for a certain growth pattern. Work from this laboratory has shown that the amount of polyosomal polyadenylated mRNA increases by more than 2-fold during the first 24 h after partial hepatectomy (2). However, the sequence complexities of nuclear RNA and of polyadenylated polyosomal mRNA in 12-h regenerating liver are no different from those of sham-operated rats (3, 4), and polyosomal mRNA populations of 16-h regenerating livers are qualitatively similar to those of normal livers (5). These findings suggest that the large changes in the amounts of mRNA and the modifications in polysome size, which take place at the early stages of liver regeneration, might not be accompanied by major qualitative alterations in polyosomal mRNA populations. In this paper, we report studies on the percentage of the genome expressed, the complexity of polyosomal polyadenylated mRNA, and the homology between mRNA populations using mRNA preparations obtained from livers at various stages of regeneration after partial hepatectomy and livers from sham-operated rats.

MATERIALS AND METHODS

Animals—Male albino rats (Holtzman strain, Charles River Breeding Laboratories) were used for all experiments. The animals were kept in temperature-controlled rooms with 12-h alternating light and dark cycles. Weanling animals weighed 50 to 70 g at the time of the experiment, but were not fasted. All surgical procedures were done under continuous oxygen-ether anesthesia as previously described (7). To deplete liver glycogen, food was withdrawn 14 h before killing the rats between 9 and 11 a.m. In experiments involving weanling rats, to improve the synchronization of DNA synthesis, food was removed at 21 p.m. (after their first night feeding) and the animals were killed the following morning at 9 a.m. (8).

Isolation and Characterization of Polyosomal Poly(A)+ mRNA—The methods for obtaining polyosomes and for the isolation of poly(A)+ RNA by chromatography on poly(U) Sepharose were those previously described (4). After affinity chromatography, the polyadenylated RNA was precipitated with alcohol, redissolved in a small volume of 10 mM NaCl, and passed through a Chelex 100 (Bio-Rad Laboratories) column. The poly(A) content of the purified poly(A)+ mRNA was determined by hybridization with [3H]poly(U) using a 15-fold excess of [3H]poly(U) to poly(A) exactly as previously described (2). The concentration of mRNA present in the preparations was calculated by assuming that, on the average, the poly(A) tract constitutes approximately 5.5% of the mRNA molecule (2). The
average length of the poly(A)* mRNA, determined by sucrose gradient centrifugation under denaturing conditions, was approximately 1800 nucleotides for preparations from both sham-operated and regenerating livers (2).

Preparation and Characterization of [3H]cDNA—Synthesis of [3H]cDNA in vitro, using avian myeloblastosis virus reverse transcriptase, was performed by the procedure described by Colber et al. (4) as modified by Atryzek et al. The reaction was carried out for 45 min at 45°C in a 20-μl incubation mixture containing: 3 μg of polysomal poly(A)* RNA, 0.5 mM each dTTP, dGTP, dATP, 50 mM Tris (pH 8.3), 20 mM dithiothreitol, 12 mM magnesium acetate, 30 μg/ml of oligo(dT)12.5 (Collaborative Research), 100 μg/ml of actinomycin D, 125 μCi of [3H]CTP (Amersham, specific activity, 12 Ci/mmol) or New England Nuclear, specific activity, 48 Ci/mmol, and 5 units of avian myeloblastosis virus reverse transcriptase (gift from Dr. J. W. Beard, Life Sciences, Inc., St. Petersburg, Fla.). After extraction of the incubation mixture with an equal volume of chloroform-isoamylic alcohol (24:1), the final aqueous phase was passed through a column containing Sephadex G-50 over Chelex 100. The excluded fractions were pooled, treated with base (0.5 N NaOH, final concentration), boiled for 5 min, and precipitated in alcohol after the addition of NaCl (0.2 M, final concentration) and 50 μg/ml of tRNA. The sizes of cDNA were determined by electrophoresis in 3.5% polyacrylamide gels cross-linked with N,N'-diallyl tartardiamide (9) and containing 7.0 M urea, using Eco RI restriction fragments of ϕX174 (Bethesda Research Laboratories) as markers (4). After staining with ethidium bromide to visualize the markers, the gel slices were washed and each fragment was eluted in 2% periodic acid for determination of radioactivity corresponding to DNA-DNA duplexes.

Messenger RNA cDNA Hybridization—The hybridization reactions were carried out at 70°C in 0.24 M sodium phosphate buffer (pH 6.9), 1 mM EDTA, and 0.05% sodium dodecyl sulfate in sealed, sterile, siliconized capillary tubes. The tubes were boiled for 5 min and incubated for the time necessary (generally less than 24 h but not longer than 94 h) to reach the appropriate Rd value (Rd is the product of the concentration of RNA nucleotides in moles/liter and time in seconds). The extent of hybridization was determined by digestion with nuclease S1 from Aspergillus oryzae (Boehringer Mannheim) (4). Zero time values for the hybridization (1 to 3%) were used as background and have been subtracted from all data presented.

Hybridization of [3H]-labeled Single Copy DNA with Nuclear RNA—The methods for the preparation of labeled single copy rat DNA, rat liver nuclear RNA, and the conditions of hybridization have already been described in detail (3).

RESULTS

The overall sequence of events during liver regeneration in rats is well known: an initial phase of hypertrophy lasting approximately 14 h during which the rate of replication is not altered, is followed by a period characterized by a marked increase in DNA synthesis which reaches a peak 22 and 24 h after partial hepatectomy in weanling and young adult rats, respectively (1, 10, 11). A major wave of mitosis follows the peak of DNA synthesis by about 10 h. By 72 h, the mass of the regenerating liver has increased approximately 2.5-fold and the DNA content is almost the same as that of the intact organ. Thus, the times selected for study (12, 24, and 72 h after partial hepatectomy) represent the phases of hypertrophy, hyperplasia, and restoration of function with diminished growth rate.

Hybridization of Single Copy DNA with Nuclear RNA from Normal and 24-h Regenerating Liver—The curves for the hybridization of nuclear RNA from livers of sham-operated and partially hepatectomized rats (killed 24 h after the operation) with [3H]-labeled single copy rat DNA are shown in Fig. 1. The fraction of DNA hybridized was determined by S1 nuclease digestion of the incubation mixtures followed by separation of the hybrids by Sephadex G-100 chromatography as previously described (3). Using this procedure, the zero time hybridization background levels are reduced to less than 1%. The proportion of the hybrids at each Rd value which corresponds to DNA duplexes was determined by testing the incubation mixtures with RNases A and T1. These values are shown by the dashed lines in Fig. 1. To calculate the percentage of radioactivity in DNA-RNA hybrids, shown by the solid lines in Fig. 1, the amount of label corresponding to DNA duplexes was subtracted from that of the total hybrid. These lines represent the best fit to the data points obtained by computer analysis (3). The two curves have identical levels of saturation corresponding to approximately 4.5% of the single copy DNA. After correcting this value for the fraction of DNA in the preparation which does not react, we estimate that approximately 12% of the single copy genome is transcribed in the livers of sham-operated and partially hepatectomized rats 24 h after the operation, assuming asymmetric transcription. This value agrees with that calculated for 12-h regenerating liver (3) and with the data of Wilkes et al. (5).

Sequence Complexity of mRNA during Liver Regeneration—As several laboratories, including our own (4, 5, 10, 11) have reported, the hybridization of rat liver polyadenylated polysomal mRNA with its homologous cDNA spans approximately 5 log units on a Rd scale as shown in Fig. 2. This indicates the presence of mRNA species distributed over a wide range of concentrations. Using the computer analysis developed by Pearson et al. (15) for nonlinear least squares fit to the data, acceptable curves can be obtained in which the mRNA populations are divided into 2 to 4 frequency classes. In the conventionally used three-component analysis (16), the polyadenylated polysomal mRNA populations from livers of rats at 24 and 72 h after sham operation are distributed in

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FIG. 1. Hybridization of [3H]-labeled single copy DNA with nuclear RNA from sham-operated (C) and regenerating livers (O), 24 h after the operations. Nuclear RNA (concentrations of 2 to 4 mg/ml) was mixed with [3H]-labeled single copy DNA at a ratio of approximately 10. Conditions of hybridization and the procedures for determining the fraction of DNA hybridized are described under "Materials and Methods." Each point represents RNA-DNA hybrids developed by Pearson et al. (15) for nonlinear least squares fit to the data, acceptable curves can be obtained in which the mRNA populations are divided into 2 to 4 frequency classes. Using the computer analysis developed by Pearson et al. (15) for nonlinear least squares fit to the data, acceptable curves can be obtained in which the mRNA populations are divided into 2 to 4 frequency classes.
classes which comprise approximately 20% of the total for the most abundant sequences, 35% for intermediate, and 25% for the rare sequences. Polysomal mRNA populations from 24- and 72-h regenerating livers have very similar frequency distributions. This is illustrated in Fig. 2 and the analysis of these curves is presented in Table I. The sequence complexity of mRNA from 24-h regenerating liver is very similar to that of mRNA of sham-operated animals. It should be noted that at this time, the amount of polysomal polyadenylated RNA in regenerating liver is approximately 2.5-fold higher than that of normal rats (2). The computer-calculated complexity values for mRNA of 72-h regenerating liver (Table I) are higher than those of the 24-h group. However, a detailed analysis of the computer estimates, as well as the results of the heterologous hybridizations shown below, suggest that the higher sequence complexity estimates are a consequence of shifts in the frequency of transcripts which already existed in the population.

Homology between mRNA Populations in Normal and Regenerating Livers—Even when the sequence complexity of two mRNA populations is the same, this does not imply that the sequences present in one population are entirely homologous to those of the other. The sequence complexity is only an estimate of the total number of different sequences of average size present. Thus, mRNA populations which are qualitatively different may have the same sequence complexity. In addition, sequence complexity calculations done by kinetic analysis of hybridization curves may reflect changes in the frequency of certain transcripts rather than an absolute change in the total number of different RNA species present in the population. To determine the extent of the homology between mRNA from regenerating liver and that of livers of sham-operated rats, we performed heterologous hybridizations between polysomal mRNA from sham-operated rats and [3H]cDNA synthesized from regenerating liver mRNA. The reciprocal reactions, that is the hybridization of mRNA from regenerating liver with [3H]cDNA from livers of sham-operated rats, were also done. By comparing these heterologous reactions with the corresponding homologous curves, it is possible to determine whether regenerating liver has sequences which are not present in livers of sham-operated rats or, conversely, if livers of sham-operated animals contain mRNA sequences which are absent from regenerating livers.

We have previously reported that mRNA from 12-h regenerating livers was not completely homologous to that obtained from sham-operated rats (4). Given the results presented above, we restudied the characteristics of mRNA populations of 12-h regenerating livers. Using a much more extensive series of animals than in our original studies, mRNA from sham-operated rats, we performed heterologous hybridizations between polysomal mRNA from sham-operated rats and [3H]cDNA synthesized from regenerating liver mRNA.

The heterologous hybridization curves of mRNA from 24- and 72-h regenerating livers are presented in Fig. 3, a to c. The corresponding homologous curves (that is a curve in which the same cDNA is hybridized with its template mRNA) are shown by the dashed lines (a to c). Since the saturation levels of the heterologous curves are, in all cases, very similar or identical with those of the homologous reactions, we conclude that within the limits of the methods used, there are no obvious qualitative differences between mRNA populations of sham-operated and regenerating livers, 24 and 72 h after the operations.

Table I

<table>
<thead>
<tr>
<th>Hybridization</th>
<th>Class</th>
<th>Fraction cDNA hybridized</th>
<th>R_{A_{24}} (observed)</th>
<th>R_{A_{72}} (corrected)</th>
<th>Sequence complexity</th>
<th>Copies of each sequence per cell</th>
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<tr>
<td>Sham-operated rats</td>
<td>I</td>
<td>0.213</td>
<td>0.034</td>
<td>0.0067</td>
<td>14</td>
<td>2425</td>
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<td>II</td>
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<td>0.542</td>
<td>0.220</td>
<td>355</td>
<td>153</td>
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<td></td>
<td>III</td>
<td>0.285</td>
<td>13.96</td>
<td>7.25</td>
<td>7656</td>
<td>6</td>
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<tr>
<td>24-h Regenerating liver</td>
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<td>0.196</td>
<td>0.043</td>
<td>0.0097</td>
<td>16</td>
<td>5367</td>
</tr>
<tr>
<td></td>
<td>II</td>
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<td>0.750</td>
<td>0.312</td>
<td>504</td>
<td>306</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0.297</td>
<td>17.27</td>
<td>6.07</td>
<td>9790</td>
<td>13</td>
</tr>
<tr>
<td>72-h Regenerating liver</td>
<td>I</td>
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<td>0.0395</td>
<td>0.0102</td>
<td>16</td>
<td>3000</td>
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<tr>
<td></td>
<td>II</td>
<td>0.380</td>
<td>0.361</td>
<td>0.332</td>
<td>526</td>
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<tr>
<td></td>
<td>III</td>
<td>0.365</td>
<td>31.12</td>
<td>9.48</td>
<td>14819</td>
<td>3</td>
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</tbody>
</table>

* Corrected R_{A_{72}} = R_{A_{72}} of component class if that class was present alone.

Sequencing complexity is the number of different sequences of average nucleotide length present in the population. Mouse globin mRNA was used as a standard.

Number of copies per cell = number of copies of each sequence in the class per cell calculated according to the formula (17).

Poly(A)+ mRNA/cell x fraction of cDNA hybridized x 6 x 10^10

Base sequence complexity of RNA class x RNA size (Daltons)

= number of copies per cell

The number of nuclei per g of liver was estimated as 1.2 x 10^10 and the polysomal polyadenylated mRNA values used were 0.116 pg, 0.921 pg, and 0.161 pg per cell for sham-operated, 24- and 72-h regenerating livers, respectively (2). No corrections were made for changes in the number of binucleate hepatocytes during regeneration.

* Hybridization of polysomal mRNA from sham-operated rats to its homologous [3H]cDNA (data from Fig. 2, A——A).

* Hybridization of polysomal mRNA from 24-h regenerating liver to its homologous [3H]cDNA (data from Fig. 2, A——A).

* Hybridization of polysomal mRNA from 72-h regenerating liver to its homologous [3H]cDNA (data from Fig. 2, A——A).
FIG. 3. Hybridization of polysomal poly(A)+ mRNA from adult rat livers to heterologous [3H]cDNAs. See legend to Fig. 2 for hybridization conditions and notation. Homologous curves for each [3H]cDNA are included for comparison. a, hybridization of 24-h sham [3H]cDNA to its template mRNA (A - - A, saturation level = 84.0%) and to mRNA from 24-h regenerating liver (● - - ●, saturation level = 81.3%); b, hybridization of 24-h hep [3H]cDNA to its template mRNA (A - - A, saturation level = 84.8%) and to mRNA from livers of sham-operated rats obtained 12 h after the operation was hybridized with [3H]cDNA transcribed from polysomal polyadenylated mRNA from 12-h regenerating liver. The results are presented in Fig. 3d in conjunction with the homologous reaction between 12-h regenerating liver mRNA and its [3H]cDNA. The saturation levels of both reactions are practically identical, and the curves do not indicate qualitative differences between mRNA populations of 12-h regenerating liver and those of the controls. Thus, we were unable to confirm the previously reported data.

Homology between mRNA Populations from Regenerating Livers of Weanling Rats and Those of Sham-operated Animals—The proportion of cells which replicate their DNA at the peak of DNA synthesis in the regenerating liver of weanling rats is approximately 2-fold higher than that of adults (11). The synchronization of the process can be further improved by feeding the animals immediately before the start of the starvation period (8). If new mRNA sequences appear in regenerating liver in conjunction with the burst of DNA synthesis, it is likely that these sequences would be more easily detected in weanling animals than in sham-operated rats obtained 12 h after the operation was hybridized with [3H]cDNA transcribed from polysomal polyadenylated mRNA from 12-h regenerating liver. The results are presented in Fig. 3d in conjunction with the homologous reaction between 12-h regenerating liver mRNA and its [3H]cDNA. The saturation levels of both reactions are practically identical, and the curves do not indicate qualitative differences between mRNA populations of 12-h regenerating liver and those of the controls. Thus, we were unable to confirm the previously reported data.

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FIG. 4. Hybridization of polysomal poly(A)+ mRNA from regenerating livers of weanling rats to homologous and heterologous [3H]cDNAs. Upper diagram, hybridization of 22-h hep [3H]cDNA from weanling rats to its template mRNA (A - - A, saturation level = 81.0%) and to polysomal mRNA from livers of 24-h sham-operated adult rats (● - - ●, saturation level = 84.7%); lower diagram, hybridization of 24-h hep [3H]cDNA from adult rats to its template mRNA (A - - A, saturation level = 84.0%) and to polysomal mRNA from 22-h regenerating liver of weanling animals (● - - ●, saturation level = 88.0%). See legend to Fig. 2 for conditions of hybridization and notation.
were then transferred to another water bath, kept at the appropriate temperature required for the melting curve (73-100°C), and incubated for 5 min. The percentage of duplexes remaining at each temperature was determined by digestion with nuclease S1. Although there are small variations between homologous and heterologous reactions at the lower temperature ranges, the curves are identical for temperatures between 82-100°C and have a Tm of 92°C. Thus, there is good fidelity of matching between mRNA and cDNA in both homologous and heterologous reactions.

**DISCUSSION**

It has been generally assumed that genomic "derepression" takes place at the early stages of liver regeneration. Most of these assumptions are based on reports indicating that the capacity of total liver RNA to hybridize with unfractionated DNA doubles 1 h after partial hepatectomy (18). However, as we pointed out earlier (4, 19, 20), and as discussed recently by Miller and McCarthy (21) and Wilkes et al. (5), the methods used in the original reports (18) measured only the hybridization of repetitive DNA sequences. Total RNA preparations usually contain less than 5% mRNA, and since the RNA labeling was done in vitro (18), the proportions of mRNA and rRNA present in the various preparations could not be calculated. Moreover, the early experiments were done with total RNA:DNA ratios of approximately 1 and it is now known that an RNA excess of at least 500-fold is necessary for this type of hybridization reaction. Previous work from this laboratory demonstrated that when the appropriate RNA:DNA ratios are used in these reactions and all types of RNA are labeled in vitro to the same specific activity, obvious differences between RNA populations of normal and regenerating liver are not observed (19, 20).

More recently, our laboratory and others have examined the diversity and extent of homology between polyadenylated mRNAs in normal and regenerating livers and the percentage of the nonrepetitive genome transcribed in these experimental conditions (3-5, 22, 23). In this communication, we show that the sequence complexities of polyadenylated polyosomal mRNA at various stages of liver regeneration are very similar to those of mRNA from livers of sham-operated controls. Moreover, with the methods described in this paper, we did not find qualitative differences between polyadenylated polyosomal mRNA populations of 12-, 24-, and 72-h regenerating livers, and those of livers of matched sham-operated rats. In addition, we did not detect qualitative dissimilarities between mRNA populations of regenerating livers of weanling rats, which have a better synchronized and enhanced regenerative response, and those of sham-operated animals. Thus, using the type of analysis described in this report, we were unable to detect "new" sequences, that is sequences present only in the polysomes of regenerating livers. We had concluded, in a previous report, that 12 h after the operation the sequence complexities of polyadenylated mRNA from partially hepatectomized and sham-operated rats are similar but that these two populations did not appear to be entirely homologous. In the more extensive studies reported here, we have confirmed that the sequence complexities of these two mRNA preparations are very similar. However, we could not detect any sequences specific for liver from either partially hepatectomized or sham-operated rats. Wilkes et al. (5), in a detailed study of events taking place in 16-h regenerating liver, found no qualitative differences between polyadenylated mRNA from normal and regenerating livers. These authors also point out that alterations in the frequency distribution of polyadenylated mRNA sequences exist in 16-h regenerating liver.

Although error estimates of the computer calculations of RNA sequence complexities from hybridization curves are relatively small, complexity estimates which differ by 2-fold or less for two different mRNA populations cannot be accepted as valid without further proof. Since heterologous hybridizations showed no differences between polyosomal polyadenylated mRNA populations from livers of sham-operated rats and regenerating livers, the higher sequence complexity estimates found in 72-h regenerating liver mRNA preparations are probably a consequence of changes in the frequency of existing sequences rather than the appearance of entirely new species.

Wilkes et al. (5) have shown that polyosomal polyadenylated mRNA sequences which were abundant in normal liver became less frequent in 16-h regenerating liver. Similarly, Miller and McCarthy (21) have suggested that only changes in the abundance of mRNA sequences take place in mouse kidney during compensatory growth despite their finding of an almost 2-fold increase in the complexity of mRNA from the hyperplastic organ. Such differences in mRNA frequency might be the result of differential transport of sequences from nucleus to cytoplasm as indicated by the studies of Siegel et al. (24) and others (3-5, 25-27). Our experiments with regenerating livers of weanling animals suggest that polyosomal mRNA sequences which exist in the liver before the operation, become less abundant at the time of maximal DNA synthesis. On the other hand, more complex frequency changes appear to take place in the 24-h regenerating liver of adult rats since heterologous reactions using cDNA synthesized from liver polysomal mRNA from either sham-operated or partially hepatectomized rats were slower than the corresponding homologous reactions.

Although in mixing experiments, nuclear RNAs from normal and regenerating livers appear to be completely homologous to each other, Wilkes et al. (5) and Krieg et al. (22) reported that approximately 10% of the sequences in 12- or 16-h regenerating liver polyadenylated nuclear RNA were not found in normal livers. Our experiments support the view that these sequences are confined to the nucleus as suggested by Wilkes et al. (5). Although the origin of these nuclear sequences remains to be established, they may represent adenylate of pre-existing nonadenylated sequences, a mechanism which may also occur in polyosomal mRNA of regenerating livers (2).

A variety of systems has been studied so far, but little evidence exists for the transcription of a substantial number of entirely new sequences during growth processes (3-5, 17, 21, 25, 28-30). To date, the evidence suggests that the changes in mRNA transcripts are quantitative rather than qualitative in nature. However, hybridization techniques are limited in their sensitivity, and thus, differences between mRNA populations involving a few sequences could remain undetected, especially if these messengers belong to the rare frequency class. Nevertheless, the hybridization results suggest that the normal liver cell already contains most of the polyosomal polyadenylated sequences necessary for growth and replication and that only adjustments in the amounts or expression of these sequences are needed for these processes.

After this paper was submitted, Grady et al. (23), using a hybridization method of very high sensitivity, showed that total cell RNA from normal livers contain all of the sequences complementary to nonrepetitive DNA found in 24- and 48-h regenerating liver. However, the regenerating liver preparations lack some sequences present in the intact organ.

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REFERENCES

Gene expression and the diversity of polysomal messenger RNA sequences in regenerating liver.
C A Scholla, M V Tedeschi and N Fausto