Polyribosomes in Rat Tissues

II. The Polyribosome Distribution in the Minimal Deviation Hepatomas

THOMAS E. WEBB, GÜNTER BLOBEL, VAN R. POTTER, AND HAROLD P. MORRIS

McArdle Laboratory for Cancer Research, Medical School, University of Wisconsin, Madison, Wisconsin, and the Laboratory of Biochemistry, National Cancer Institute, NIH, Department of Health, Education and Welfare, Bethesda, Maryland

SUMMARY

In agreement with the concept of the "minimal deviation" tumor, the fraction of bound polyribosomes in the postmitochondrial supernatant was 60–70% in normal and regenerating adult liver, 40% in Hepatomas 7787 and 7800, 20% or less in several other hepatomas, and approximately 0 in immature liver and in the Novikoff hepatoma. In the hepatomas the proportion of the bound polyribosomes appears to correlate with their degree of differentiation and inversely with their growth rate. All hepatomas examined appear to have a high concentration of free dimers, which is revealed in the abnormal C-ribosome and total ribosome patterns.

The possible physiologic significance of those polyribosomes operationally defined as "free" is of considerable interest and may be related to the aberrant responses to factors that control enzyme synthesis in normal liver.

METHODS

Details concerning the housing and feeding of the rats were similar to those given previously (42). Normal rat liver was usually obtained from Holtzman rats (Holtzman Co., Madison, Wis.). The Morris hepatomas 5123C, 7787, 7793, and 7794A (18, 19) showed characteristically higher monomer and dimer peaks than normal liver; the relative concentration of the heavier polyribosomes varied with the tumor and the subline. Furthermore, 80–100% of the C-ribosomes (polyribosomes) were recovered from these tumors without DOC treatment, in contrast to normal liver, where the recovery was always less than 50%.

A similar analysis has been applied to additional minimal deviation hepatomas. The results offer further support for the concept of the minimal deviation tumor (31, 32) and suggests that there may be structural defects in the enzyme-forming system, which is assumed to involve the endoplasmic reticulum (28) in addition to messenger RNA (mRNA), ribosomes, and amino acid transfer RNA.

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2 Present address: Department of Biochemistry, The Medical School, University of Manitoba, Winnipeg, Manitoba, Canada.

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peak in normal adult rat liver, and where \( A_0 \) and \( A_n \) are equivalent polyribosome concentrations. The \( A_n \) is to the left and right, respectively, of this and the subsequent charts.

As noted previously (42), the C-ribosomes contain only a fraction of the monomers and dimers originally present in the \( S_2 \). In the present work the total ribosomes (i.e., monomers, dimers, and heavier polyribosomes) present in the crude \( S_2 \) were roughly analyzed in a manner similar to that used for the purified C-ribosomes, but with the substitution of an exponential gradient increasing from 10% to 30% sucrose (39). In this case the syringe serving as reservoir for 40% sucrose in the gradient machine had a cross-sectional area twice that of the syringe used as the mixing chamber (2). The gradient with the overlaying sample was centrifuged for 3 hr, and the effluent was monitored at 265 nM. All sucrose solutions used in the preparation and analysis of the polyribosomes were prepared in 0.05 M Tris(hydroxymethyl)aminomethane, 0.025 M KCl, and 0.005 M MgCl\(_2\), pH 7.5.

As before (42) the \( n_0/n_r \) and \( -DOC/+DOC \) ratios are used to report significant findings. The \( n_0/n_r \) ratios as calculated from the C-ribosome patterns are used to estimate the deviation from normal rat liver (after an overnight fast) of the concentration of any particular species containing \( n \) ribosome units. Thus \( n_0 \) and \( n_r \) are the relative heights of the experimental tissue and normal liver respectively, after both patterns have been corrected to equivalent polyribosome concentrations. More specifically, \( n_0/n_r = n_r (n_0 A_0/A_n) \), where \( n_r \) is the height of peak \( n \) in normal adult rat liver, and where \( A_0 \) and \( A_n \) are the areas of the polyribosome region (\( n \) equal to, or greater than, 5) in the optical density (O.D.) tracings of normal liver and the experimental tissue, respectively. The \( -DOC/+DOC \) ratio likewise provides an estimate (cf. Ref. 42) of the fraction of polyribosomes that are bound to the endoplasmic reticulum and the fraction that is operationally defined as “free.” Aliquots of the \( S_2 \) both with and without DOC treatment are centrifuged through a discontinuous gradient of 0.5 M/2.0 M sucrose under conditions identical to those used in the preparation of the C-ribosomes. The ratios are calculated from the O.D. or ribonucleic acid analysis (4) of aliquots of the solution of C-ribosomes after resuspension of the pellets.

RESULTS

The C-ribosome patterns of the Morris hepatomas 7787 and 7800 prepared with (+DOC) and without (-DOC) deoxycholate pretreatment of the postmitochondrial supernatant (\( S_2 \)) are shown in Charts 1 and 2. The -DOC pattern represents the distribution of that fraction of the C-ribosomes which are operationally defined as “free”: the dotted line in the +DOC pattern represents the approximate distribution of the polyribosomes that are firmly bound to the endoplasmic reticulum, as determined by the difference between the 2 curves. It is apparent that a significant fraction of the polyribosomes is firmly bound in these transplantable hepatomas in contrast to the tumors studied previously (42).

The abnormally high monomer and dimer peaks characteristic of the C-ribosome patterns of all hepatomas studied thus far are also seen in these 2 minimal deviation hepatomas. Since these ribosomal species are in relatively low concentration in the C-ribosomes of normal rat liver, they were interpreted (42) to be the result of some modification in the tumor.

The total ribosome pattern for normal liver and Hepatomas 7787, 7793, and 7794A, obtained by layering the crude \( S_2 +DOC \) of these tissues directly over an exponential gradient, is shown in Charts 3 and 4. In contrast to the purified C-ribosomes, all of the monomers are recovered in this crude system. The ultraviolet-absorbing material at the top of the gradient is made up of hemoglobin, ferritin, and soluble components of the cytoplasmic fraction of the cell.

In spite of the relatively poor resolution, the polyribosome complement and monomer peak of normal liver can be easily distinguished in Chart 3 (curve \( a \)). If the \( S_2 +DOC \) is treated with ribonuclease, the polyribosomes are broken down and most of the ultraviolet-absorbing material appears in the monomer peak (curve \( b \)).

The “total ribosome” patterns for Hepatomas 7787 (curve \( a \)), 7793 (curve \( b \)), and 7794A (curve \( c \)) are shown in Chart 4. When compared with the patterns for normal rat liver there is again a significant difference in the hepato...
toma patterns, mainly with respect to the high dimer peak. The high dimer peak was also present in the total ribosome pattern of Hepatoma 7787 when the DOC treatment was omitted (not shown); the distribution of the heavy polyribosomes was masked in such instances by the sedimenting endoplasmic reticulum. This aberrant pattern appears to persist in Hepatoma 7793 at high dilutions (curve b). It is of significance in this respect that the \( n_0/n_r \) ratios where \( n = 1 \) or \( n = 2 \) for the C-ribosome pattern of tumors remain relatively constant over a 4-fold range of dilution of the \( S_2 \). These results suggest that the abnormally high dimer peak originally detected in the C-ribosome patterns is not an artifact originating during the preparation of the latter but probably represents the \textit{in vivo} situation. The concentration of monomers does not, however, appear to be exceptionally high in the hepatomas. The total ribosome pattern for Hepatoma 7794A (Chart 4c) confirms the earlier finding (42) of the relative paucity of polyribosomes in this tumor.

The \(-\text{DOC}/+\text{DOC}\) ratios and \( n_0/n_r \) ratios for normal and regenerating liver and several hepatomas are tabulated in Table 1. As found previously (42), the \(-\text{DOC}/+\text{DOC}\) ratio for normal liver (after an overnight fast) is always less than 0.5 and is usually within the range 0.30–0.40. Approximately the same ratio is found in 12-hr regenerating liver. The Morris hepatomas 7787 and 7800 consistently gave ratios of 0.60 (±0.02), respectively. Recent estimates of the \(-\text{DOC}/+\text{DOC}\) ratio for Hepatomas 7793 and 5123C, with a few exceptions, give a value of approximately 0.8 (based on 8 animals), rather than 1.0, as reported previously (42). This difference is attributed to (a) better resuspension of the C-ribosome pellet when water is used as the medium and (b) omission of the low-speed centrifugation of the resuspended pellet.

Hepatoma 5123C, like subline A (cf. Ref. 42), appears to have slightly less than 20% of its polyribosomes strongly bound; the bound polyribosomes in the Reuber (H-35) hepatoma is also of this order. In contrast, most of the polyribosomes appear to be free in the Novikoff hepatoma. The data suggest that all the hepatomas examined have a high \(-\text{DOC}/+\text{DOC}\) ratio as compared with normal liver. As indicated in Table 1, the \(-\text{DOC}/+\text{DOC}\) ratio for the embryonic and the newborn rat liver is approximately 1.0; thus all of the polyribosomes of the \( S_2 \) of immature rat liver are operationally free. Protein of unknown origin contaminates the C-ribosome pellet obtained from the \( S_2 \) – DOC of immature rat liver, which results in high protein/RNA ratios and \(-\text{DOC}/+\text{DOC}\) ratios based on O.D. that are significantly greater than 1.0. The change in the \(-\text{DOC}/+\text{DOC}\) ratio during the maturation of rat liver will be presented elsewhere (G. Blobel, to be published).

**TABLE 1**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>(-\text{DOC}/+\text{DOC})</th>
<th>( n_0/n_r ) for 2 Equals 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O.D.</td>
<td>RNA</td>
</tr>
<tr>
<td>1. Normal adult liver</td>
<td>0.34 (63) (0.15–0.45)</td>
<td>0.39 (12) (0.25–0.46)</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>0.38 (16) (0.21–0.52)</td>
<td>0.42 (2) (0.41–0.43)</td>
</tr>
<tr>
<td>(12 hr postoperative)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Hepatoma 7787</td>
<td>0.61 (12) (0.50–0.70)</td>
<td>0.60 (6) (0.53–0.70)</td>
</tr>
<tr>
<td>3. Hepatoma 7800</td>
<td>0.57 (5) (0.55–0.59)</td>
<td>0.55 (5) (0.54–0.56)</td>
</tr>
<tr>
<td>4. Hepatoma 7793</td>
<td>0.80 (9) (0.73–0.85)</td>
<td>0.82 (4) (0.80–0.86)</td>
</tr>
<tr>
<td>5. Hepatoma 5123C</td>
<td>0.83 (2) (0.82–0.84)</td>
<td>0.83 (2) (0.75–0.90)</td>
</tr>
<tr>
<td>6. Reuber hepatoma</td>
<td>0.89 (4) (0.85–0.92)</td>
<td>0.83 (4) (0.80–0.90)</td>
</tr>
<tr>
<td>H-4 (tissue culture)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7. Novikoff hepatoma</td>
<td>1.00 (8) (0.99–1.10)</td>
<td>1.00 (5) (0.96–1.18)</td>
</tr>
<tr>
<td>Novikoff N1-S1 (tissue</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(tissue culture)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>8. Immature rat liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryonic (18 days)</td>
<td>1.40 (3) (1.00–1.55)</td>
<td>1.10 (3) (0.98–1.15)</td>
</tr>
<tr>
<td>1 day old</td>
<td>1.03 (3) (0.98–1.06)</td>
<td>0.91 (3) (0.88–1.00)</td>
</tr>
</tbody>
</table>

* The abbreviations used are: DOC, deoxycholate; O.D., optical density; RNA, ribonucleic acid; and \( S_2 \), postmitochondrial supernatant.

* Shown for each tissue are: (a) the average \(-\text{DOC}/+\text{DOC}\) ratios based on O.D. and RNA analysis and qualified in parentheses by the number of rats and the range of values and (b) the \( n_0/n_r \) ratios of the dimer peak calculated from the size distribution of C-ribosomes purified from the \( S_2 + \text{DOC} \).
It can be concluded from the \( n_p/n_r \) ratios (Table 1) that the high dimer peak is a characteristic of all the hepatomas, even after transfer to tissue culture. For example, high ratios are found in the C-ribosomes of the Reuber hepatoma (H-4 cells) and Novikoff hepatoma (N1-S1 cells) grown in tissue culture.

**DISCUSSION**

The present data and those presented previously (42) suggest that the fraction of "bound" polyribosomes in the S$_2$ of hepatomas varies from approximately 0 in the Novikoff hepatoma to about 40% in the minimal deviation Hepatomas 7787 and 7800; the other hepatomas studied fall between these extremes. Although normal adult liver has 60–70% of the polyribosomes bound, the present data (cf. Ref. 24) suggest that most of the polyribosomes are free in immature rat liver. Thus the −DOC/+DOC ratio for the Novikoff hepatoma resembles that of newborn rat liver, whereas the ratios of Hepatomas 7787 and 7800 are closer to the ratio of adult liver. These findings are reasonable in terms of the "minimal deviation" concept (31–34), which suggests that the biochemical and morphologic deviations of such tumors (e.g., hepatomas) from the cell of origin (e.g., the differentiated parenchymal cell) are fewer than in any previous demonstrated instance. The biochemical and morphologic parameters are usually based on the status of the marker enzymes and organelles typical of the cell of origin.

The −DOC/+DOC ratios for the Novikoff hepatoma and Hepatoma 7787 correlate well with published data concerning their biochemical (6, 30) and morphologic (6, 16) differentiation. The storage of glycogen in Hepatoma 7787 is in itself strong evidence for its high degree of differentiation. Two out of 3 sublines of Hepatoma 5123, Hepatoma 7793, and Hepatoma H-35 have a measurable fraction of bound polyribosomes; these tumors are also known to have several marker enzymes (7, 18, 29, 34, 43) and moderate amounts of endoplasmic reticulum (6, 7, 18). Insufficient morphologic data are available concerning Hepatoma 7800, although the available biochemical data (19, 34) suggest that it is quite highly differentiated.

Since a correlation has been found between the degree of ultrastructural differentiation and the growth rate of several hepatomas (6), it might be expected that the −DOC/+DOC ratios will also bear a similar relationship to the growth rate. In general those hepatomas which have a high ratio have a rapid growth rate, whereas those with a low ratio grow more slowly. For example, Hepatomas 7787 and 5123 have very slow and moderate growth rates, respectively; the former has a transplant generation time of approximately 10 months, whereas the latter has a transplant generation time of 2–3 months (cf. Ref. 6). Hepatoma 7800 appears (19) to have a growth rate that is approximately half that of Hepatoma 5123A. The Reuber hepatoma appears (19) to have a growth rate only slightly higher than Hepatoma 7800. However, a comparison between the Morris and Reuber hepatomas may not be feasible, since they are carried in different strains of rats; the growth rates of Hepatomas 5123 and 7800 are significantly modified by the sex of the host (19). The rapidly growing Novikoff hepatoma has a transplant generation time of less than 1 week (unpublished data).

Preliminary studies suggest that the Morris hepatoma 7794B, which has numerous profiles of tubular endoplasmic reticulum, but only small amounts of organized ergastoplasm, and which has a slow growth rate (6) appears to have somewhat fewer bound polyribosomes than predicted (unpublished data). Although preliminary, the results show a maximum of 28% of the polyribosomes of the 4th generation of this hepatoma to be bound. It is possible that the growth rate of this tumor is temporarily modified by hormones or other factors and will increase during subsequent transplants. There is now evidence that the 5th generation of this tumor is growing considerably faster than previous generations.

The results of earlier studies have been reviewed (13, 20, 23); these suggest that malignant and embryonic cells contain less endoplasmic reticulum than normal adult cells of the same tissue and that variations in the amount of endoplasmic reticulum may be linked to differentiation. A correlation between dedifferentiation and growth rate has been found previously (6, 12). The present data suggest that the fraction of bound polyribosomes correlates well with the degree of differentiation of the tissue. The fact that the −DOC/+DOC ratio for regenerating liver is in the range of normal liver supports the view (20) that regenerating liver represents increased growth rate with the maintenance of the differentiated state. It is probably significant in this respect that regenerating liver, but not several hepatomas, retains the cholesterol-negative feedback system (38). The possible relationship of the bound polyribosomes to the tissue specific antigen of adult liver should also be considered. The latter is localized in the microsome fraction (9) and disappears in liver cancer induced by feeding 4-dimethylaminoazobenzene to rats (44). The titer in regenerating liver is apparently half that of adult liver (8) but is very low or nil in several minimal deviation hepatomas (R. Suss, personal communication).

With the possible exception of a method based on the differential sensitivity to ribonuclease (40), the −DOC/+DOC ratio is at present the only suitable biochemical method for detecting and estimating the fraction of "free" and "bound" polyribosomes in the S$_2$ fraction of the cell. There are 2 possible limitations to the method, which should be considered. First, during the preparation of the S$_2$, which involves centrifuging at 17,300 × g$_{max}$, a significant but undetermined fraction of the rough-surfaced endoplasmic reticulum sediments with the nuclei and mitochondria (25, 36). The existence of this 2nd possible source of bound polyribosomes may explain why the −DOC/+DOC ratio for the Novikoff hepatoma is 1.0 while small remnants of the rough endoplasmic reticulum are found in electron micrographs (16); rough endoplasmic reticulum is also visible in the liver of the 1-day-old rat (22). The release of the ribosomes from this heavy microsome fraction under certain physiologic conditions would result in the modification of the normal C-ribosome and total ribosome patterns.

A second limitation of the method is that it involves, first, homogenization and, then, separation of the "free" and "bound" polyribosomes by centrifugation through a
discontinuous gradient, thereby rendering difficult the possibility of distinguishing between “free” and “loosely bound” polyribosomes. In addition to the data discussed above, the following studies suggest that the free polyribosomes are unattached to the endoplasmic reticulum in vivo and also support the suggestion that the ratio so obtained reflects the cellular differentiation of the tissue.

(a) The C-ribosome preparations are free of significant contaminating endoplasmic reticulum as judged by electron microscopy (1) and tests for glucose-6-phosphatase (unpublished data) and catalase (14). (b) The Novikoff hepatoma, which has few bound polyribosomes (cf. "Results"), has a decreased phospholipid content compared with liver, especially with respect to the microsome fraction (22). (c) Results of earlier studies suggest that most of the ribosomes in the Jensen sarcoma (26) and the Novikoff hepatoma (16, 17) and most or all of the polyribosomes in reticulocytes (10, 41) are unattached. (d) About 90% of the polyribosomes of fertilized sea urchin eggs are "free" yet active in in vivo amino acid incorporation (40). (e) In electron microscope studies of established mouse fibroblast lines (11) micrographs show structures resembling free polyribosomes in situ. The last study is of particular interest since the fibroblasts showed little endoplasmic reticulum and many structures resembling free polyribosomes during log-phase growth, whereas in the resting phase, when they actively synthesized collagen, there was good development of the rough endoplasmic reticulum. It is probable that the "clusters" or "rosettes" of free ribosomes often seen in electron micrographs of cells from many tissues are free polyribosomes. Additional support for this theory is found in electron micrographs of healthy and degenerating Sarcoma I cells (4). The former have numerous clusters of free ribosomes in their cytoplasm whereas in the latter the free ribosomes are distributed diffusely.

The presence of numerous free polyribosomes and little rough endoplasmic reticulum in the cytoplasm of rapidly growing tissues raises the question whether the former are actively engaged in protein synthesis; recent in vivo (G. Blobel, to be published) and in vitro (1) studies suggest that the free polyribosomes incorporate labeled leucine. Thus unless other organelles synthesize most of the cellular proteins, the involvement of the free polyribosomes would seem obligatory. Two theories have been advanced (cf. Refs. 13, 20) concerning the role of the rough endoplasmic reticulum in protein biosynthesis: (a) The synthesis of proteins occurs only on ribosomes that are bound to the endoplasmic reticulum. (b) Free particles are involved in the elaboration of cellular proteins whereas attached particles intervene in the synthesis of exported proteins. To these 2 possibilities may be added a 3rd, which is a modification of the 2nd: (c) Free polyribosomes synthesize proteins concerned with cell growth and proliferation, whereas bound polyribosomes synthesize proteins characteristic of the cell type (differentiated functions) whether or not they are for export. Since at least remnants of endoplasmic reticulum are found in all tumors (13), the 3rd proposal might again be modified to allow for the synthesis of some inducible enzymes essential for cellular proliferation in these fragments. The reticulocyte must be considered an exception since it does not have a nucleus to maintain an endoplasmic reticulum.

The significance of the high dimer concentration in the hepatomas remains to be determined. It is possible that the loss of endoplasmic reticulum and the presence of this peak are in some manner related. Pitot and Peraino (28) have suggested that the endoplasmic reticulum stabilizes the mRNA of the polyribosome complex and that defects in the membrane are responsible for the anomalies in enzyme induction that were invariably found in neoplastic tissues. Pitot (27) has further shown that the template half life for many inducible enzymes differs in normal and neoplastic liver. The dimer peak might then be a by-product of a destabilized template. The present results offer further support to the view that the endoplasmic reticulum is defective in the hepatomas; however, defective enzyme induction (32) and the loss of feedback in cholesterol biosynthesis (38) may also be the result of more subtle defects, not detectable by the present procedure. It is unlikely that the high dimer peak is due to decreased production of mRNA although C-ribosome patterns obtained from rats treated with actinomycin D also have a high dimer peak (39), and it has been suggested (21) that monomers free of mRNA tend to dimerize. However, this mechanism requires that the relatively high monomer peak in normal rat liver contains mRNA; yet the monomers do not incorporate amino acids (3, 21).

It can be concluded that gross dedifferentiation need not be a permanent characteristic of neoplasia. Indeed, the Morris hepatoma 7787 stores glycogen (6), the Reuber hepatoma H-35 produces bile (37), and a mouse mammary tumor is known (cf. Ref. 23) to secrete milk. Furthermore, if the temporary loss of any or all of the cytoplasmic information resident in the endoplasmic reticulum or the deletion (32) of a small part of such information is an essential attribute of neoplasia, it is probably concerned with the promotion, rather than the initiation, phase of carcinogenesis (35). It is important to determine whether the increased proportion of free polyribosomes in tumor cells is diverted from the production of proteins characteristic of the cell type (i.e., proteins of differentiation) to the production of proteins for cellular proliferation, thereby contributing in part to the increased growth rate usually accompanying dedifferentiation.

REFERENCES

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