STUDIES ON THE STRUCTURE AND FUNCTION OF RIBOSOMES FROM REGENERATING RAT LIVER

Julian Scheinbuks, Paul S. Sypherd and Kivie Moldave
Departments of Biological Chemistry and Medical Microbiology, California College of Medicine, University of California, Irvine, California, 92664.

Received September 24,1974

SUMMARY: Electrophoresis of ribosomal proteins from 1-day regenerating and sham-operated rat livers, doubly-labeled with two iodine isotopes, revealed that the relative stoichiometric ratios of proteins S6 from the 40S subunit and L5, L18 and L27 from the 60S subunit, vary significantly from those obtained with the other proteins. The ratios of the altered proteins return to normal within three days after partial hepatectomy. Polypeptide chain elongation and binding of aminoacyl-tRNA were slightly greater with regenerating preparations than with control preparation, when 80S ribosomes were used, but differences were not observed when the experiments were carried out with purified subunits and exogenous template.

INTRODUCTION

Replacement of hepatic tissue following partial hepatectomy is accomplished by rapid cellular proliferation which reaches a maximum on the second day (1). The ribosomal content of regenerating hepatocytes increases markedly prior to a substantial increase in cell division rate (2,3). During this period, there is a 50% increase in the formation of 45S precursor RNA (4), although the concentration of ribosomes per unit weight of regenerating tissue does not change (3). At the third day after hepatectomy, the ribosome content of the hepatocyte appears to drop significantly (5).

An increased level of amino acid incorporation into liver microsomes from partially hepatectomized rats was reported by von der Decken and Hultin (6). The increase in protein synthesis has been shown with both microsomal and ribosomal preparations from regenerating as compared to control livers (7-9). The difference in activity of ribosomes from regenerating liver as compared to normals was not due to shifts in the pools of free and membrane bound polysomes since the relative concentrations remained the same (5). The increase in activity observed with regenerating liver ribosomes was lost after treatment with detergent or with phospholipase A; the activity with these treated ribosomes from regenerating liver was comparable to that obtained with ribosomes from normal livers (7). Tsukada and Lieberman suggested the presence of a phospholipid that stabilized or protected polysomes (7).

The changes observed in the incorporation of amino acids into protein *in vitro* have also been detected in hepatectomized animals *in vivo* (8,10). However, Scornik (3) reported that the *in vivo* rates of protein synthesis were only 10% higher in regenerating livers, and that the relative efficiency

in the translation of endogenous messenger RNA was the same for both normal and regenerating liver when massive doses of [^{3}H]leucine were injected into mice to saturate the internal pools; the small differences in the rate of protein synthesis were attributed to the conversion of monosomes to polysomes.

The changes in the activity of ribosomal preparations observed with regenerating liver suggested a detailed examination of ribosomal functions with respect to some of the intermediary steps in protein synthesis, and of the structure of the ribosome with respect to the protein composition. This communication describes some of the structural and functional features of regenerating rat liver ribosomes, as compared to those of control or sham-operated rats.

MATERIALS AND METHODS

Purified rat liver ribosomes (11) and subunits (12, 13) were obtained from control, sham-operated and partially-hepatectomized (14) rats. Elongation factors EF-1 and EF-2 from rat liver (15), and aminoacyl-tRNAs esterified with [^{3}H]phenylalanine (16) or with labeled amino acid mixture (17) were prepared as described; the specific radioactivity of the [^{3}H]phenylalanyl-tRNA was 2,300 cpm/µg tRNA and of the [^{3}H]aminoacyl-tRNA was 2,900 cpm/µg tRNA. Ribosomes and subunits were resuspended in 50 mM Tris-HCl, pH 7.3, 4 mM $MgCl_2$, 50 mM KCl, 1 mM dithiothreitol and 0.35 M sucrose (4 mg/ml) and extracted with acetic acid (18). The ribosomal protein fractions were dialyzed against 200 volumes of 1 N acetic acid for 48 hours (against 4-5 changes of acid), lyophilized and stored at -76°. The iodination of tyrosine residues (19) in ribosomal proteins was accomplished according to a modification of the procedure of Miller and Sypherd (20). One mg of protein from each of the ribonucleoprotein preparations described above was iodinated with 500 µCi of ^{125}I or ^{131}I (Amersham Searle Corp.), in solutions containing 8.5 M urea; after 30 minutes at room temperature, the iodinated proteins were precipitated with 0.2 volumes of a solution containing 50% trichloracetic acid and 6 mM mercaptoethanol, collected by centrifugation, and washed 3 times by centrifugation with 7 M urea to remove radioactive ICl.

The labeled proteins from ribosomes and subunits were separated on polyacrylamide gels by two-dimensional electrophoresis, by modification of the procedures described by Kaltschmidt and Wittmann (21). The first dimension contained 6% acrylamide and 0.3% bisacrylamide, and measured 16 cm in length. The gels were electrophoresed at 4 mA/gel, at constant amperage, for 12 hours. After electrophoresis of the 1-dimensional gel, it was removed from its tube and polymerized onto a slab gel (12% acrylamide, 0.6% bisacrylamide), 16.5 cm wide and 20 cm long. The slabs were electrophoresed at 25 mA/slab, at

constant amperage, for 40 hours. Gels were then stained with a solution containing 1% Amido Schwartz and 7.5% acetic acid, and destained electrophoretically. Samples were cut from the center of each of the stained protein spots with a No. 2 cork borer and placed into scintillation vials. The gels were digested in the vials with 0.5 ml of 30% H_2O_2 (22) and the hydrolyzed gels were counted for ^{125}I and for ^{131}I, in 20 ml of scintillation fluor (1 liter toluene, 500 ml of 95% ethanol and 4 gm omnifluor, New England Nuclear

TABLE I

Relative Stoichiometry of Ribosomal Protein Mixtures from 1-Day Sham-Operated and 1-Day Regenerating Rat Livers [1/]

Protein number	Isotopic Ratio Sham-Operated/regenerating		Protein number	Isotopic Ratio Sham-Operated/regenerating	
	S-40S proteins	L-60S proteins		S-40S proteins	L-60S proteins
1	1.03		19	1.08	0.89
2	0.92		20	0.95	0.83
3	0.88	0.97	21	0.88	1.08
4	0.88	0.96	22	0.98	1.01
5	0.97	1.92	23	1.01	0.83
6	2.00	1.00	24	0.98	1.07
7	0.90	1.20	25	0.95	0.78
8	0.97	0.98	26	0.98	1.12
9	0.93	1.02	27	0.95	1.54
10	0.87	0.94	28	0.70	--
11	1.18	0.96	29	0.72	0.96
12	1.30	1.02	30	0.72	1.04
13	0.90	1.20	31	--	0.93
14	1.00	0.96	32	0.95	0.78
15	0.80	1.14	33	0.72	0.90
16	1.07	0.90	34	0.85	1.13
17	1.00	0.80	35	1.05	1.03
18	1.18	1.44	36		1.17

1/ Approximately 0.5 mg of iodinated ribosomal protein from regenerating liver (^{125}I) were mixed with 0.5 mg of iodinated protein from sham-operated (^{131}I) rats. S-40S proteins; 1 mg of carrier 40S protein was added to the mixture. Ratios have been normalized with respect to protein S-17. Actual relative iodination ratios varied from 1.20 to 0.42. L-60S proteins; 2 mg of carrier 60S protein were mixed with the iodinated preparation. Ratios have been normalized with respect to protein L-6. Actual ratios varied from 1.42 to 0.58. Proteins S-31 (40S proteins) and L-28 (60S proteins) possesed no detectible radioactivity. Relative ratios varying between 0.7 and 1.3 were considered unity.

Corp.), with a scintillation spectrometer. The ratio of the two isotopic iodines, and the relative ratio using proteins S17 and L6 as 1.0 were calculated for each sample.

RESULTS AND DISCUSSION

When ^{131}I- and ^{125}I-labeled ribosomal proteins from 1-day sham-operated and 1-day regenerating rat livers, respectively, were mixed and co-electrophoresed in two dimensions, several proteins were obtained which appeared to have a stoichiometric ratio different from the other proteins (Table I). The relative ratios of proteins S6 (40S) and L5 (60S) [named according to the designation used by Sherton and Wool (23)] were about 100% higher, and proteins L18 and L27 were about 50% higher, than those of most of the ribosomal proteins obtained. An increase in the relative ratio (sham-operated/regenerating) reflected a decrease in the amount of that particular protein in the regenerating liver preparation.

Protein S6 is one of the rat liver ribosomal components that was phosphorylated in vitro in the presence of rat liver cytosol and GTP, and it is of interest that a number of phosphorylated derivatives of S6 were detected as extra zones in hepatectomized preparations (24). The lower amounts of the material in the S6 region, observed in the electrophoretic patterns obtained here, could reflect the altered migration of the various phosphorylated forms of the protein. The relative stoichiometry of protein L5 is similar to that of S6. Eil and Wool (25) found that the ATP-phosphokinase from rat liver cytosol modified at least 10, as yet unidentified, 60S proteins; the GTP-phosphokinase did not catalyze phosphorylation of 60S proteins, in contrast to the results with 40S subunits where at least 4 proteins were modified. The stoichiometric ratios of proteins L18 and L27 were variable. The values presented in Table I represent the greatest observed variations from unity,although relative ratios of 1.0 have been obtained occasionally. The differences in the relative stoichiometry of those proteins that seem to be altered could reflect hepatectomy-induced changes in the amounts of those proteins from regenerating liver as compared to controls, or to derivatization of these proteins to forms that migrate to different positions than the normal proteins.

Data for "satellite" proteins of S27, for proteins S28 to S30 and for proteins L37 to L41 is not available since these components were run off the gels under conditions where maximum resolution of the proteins listed in Table I was obtained. The presence of six other 40S proteins (S30 to S35) has been noted in these gels, but proteins L1 and L2 were visible only rarely in the gels containing 60S subunit proteins.

TABLE II

Relative Stoichiometry of Some Selected Ribosomal Proteins in Mixtures from 1-Day, 2-Day and 3-Day Sham-Operated and Regenerating Rat Livers [1/]

Protein number	Isotopic Ratio; regenerating/sham-operated			
	1-Day	2-Day	3-Day	Control [2/]
S4	0.80	1.00	1.09	0.97
S6	0.40	0.52	0.84	0.90
S7	1.00	1.03	1.12	0.90
S17	1.15	0.98	1.20	0.97
S23	0.83	0.92	1.00	1.00
L5	0.56	0.68	0.97	1.04

1/ Approximately 0.5 mg of iodinated 80S ribosomal protein from regenerating liver (^{131}I) were mixed with 0.5 mg of iodinated 80S ribosomal protein from sham-operated (^{125}I)rats, and 2 mg of 80S carrier protein were added to the iodinated preparations. Ratios $^{131}I/^{125}I$, have been normalized with respect to S7 (1-Day) S4 (2-Day) and S23 (3-Day and control).

2/ Control ratios were obtained with ribosomal proteins of normal rat livers, labeled with ^{125}I and ^{131}I, and treated as described for sham-operated and regenerating livers.

Table II summarizes the results, for selected proteins, obtained with animals 1, 2, and 3 days after they were partially hepatectomized or sham-operated, as compared to normal animals. The isotopes used for the iodination of hepatectomized and sham-operated animals were reversed from that shown in Table I. In this experiment the stoichiometric ratios (regenerating/sham-operated) of 1-day post-operative proteins S6 and L5, relative to the ratio of S7, were markedly lower than those of the others (S4, S17 and S23) used for comparison, and reflected lower levels of these two proteins in regenerating liver ribosomes. The relative ratios of S6 and L5 approached the values obtained with the control preparations by the third day.

Purified 80S ribosomes or ribosomal subunits obtained from 1-day regenerating rat liver were assayed for protein synthesis, and compared to similar preparations from 1-day sham-operated animals. The 80S ribosomes contained endogenous mRNA and peptidyl-tRNA but the subunits were free of these components; protein synthesis was examined in the presence of GTP, elongation factors EF-1 and EF-2, with endogenous mRNA and a mixture of radioactive aminoacylated tRNAs (15) or with poly(U) and phenylalanyl-tRNA (16). Polymerization of amino acids with 80S ribosomes, either with endogenous template or with poly(U) was slightly greater with ribosomes from regenerating liver

TABLE III

Factor-Dependent Binding of Aminoacyl-tRNA to 80S Ribosomes and to 40S plus 60S Subunits from Control, 1-Day Sham-Operated and 1-Day Regenerating Rat Liver [1/]

Ribonucleoprotein	C.p.m. Aminoacyl-tRNA bound	
	[^{3}H]aminoacyl-tRNA	[^{3}H]phenylalanyl-tRNA
Control 80S ribosomes	2,910	1,760
sham 80S ribosomes	2,860	2,490
regenerating 80S ribosomes	3,520	5,030
control 40S + 60S subunits	---	7,810
sham 40S + 60S subunits	---	7,110
regenerating 40S + 60S subunits	---	6,950

1/ Incubations contained 20 pmoles of 80S ribosomes or 7.5 pmoles each of 40S and 60S subunits, 30 µg of labeled aminoacyl-tRNA or phenylalanyl-tRNA, EF-1 from control liver and 0.2 mM GTP; reactions with phenylalanyl-tRNA also contained 5 µg of poly(U). Incubations, in a total volume of 0.1 ml, were at 37° for 20 minutes

than from sham-operated or control livers; when the assays were performed with subunits, little or no differences were observed between preparations from the three sources. When the EF-1 and GTP-dependent binding of aminoacyl-tRNA (16, 26) was investigated, some differences were observed (Table III). Endogenous template-directed binding of a mixture of aminoacyl-tRNAs was slightly greater with 80S ribosomes from regenerating liver as compared to those from sham-operated or control livers; the poly(U)-directed binding of phenylalanyl-tRNA was also significantly greater with regenerating ribosomes. However, when purified 40S and 60S subunits were used, the binding of phenylalanyl-tRNA with regenerating, sham-operated and control preparations was similar. Whether the differences observed with 80S ribosomes, particularly with respect to the factor-dependent binding of aminoacyl-tRNA, are due to differences in the number of active ribosomes or active ribosomes with available A sites, or to the presence of ribosome-associated components that influence these reactions remains to be determined.

The large (50S) subunit Escherichia coli ribosomes contains a protein that exists in the acetylated (L7) and in the non-acetylated (L12) form. The protein (L7, L12) is required for the GTP-ase activity associated with initiation (27) and peptide chain elongation (28-30). The ratio of these two forms appears to vary during the log phase growth of the bacterium (31). Changes in the stoichiometry or the forms of various "catalytically"-active or

essential proteins could relate to the loss of ribosomal activity as cells proceed into the stationary phase. However, the alteration in the relative stoichiometry or forms of the rat liver ribosomal proteins after partial hepatectomy, did not appear to affect the catalytic centers on the ribonucleoprotein particles concerned with carrying out some of the intermediary steps in peptide chain elongation.

Acknowledgements - This work was supported in part by research grants from the American Cancer Society (NP-88) and the U.S. Public Health Service (AM-15156). The authors thank Dr. Herbert Thompson for his help and Mrs. Eva Mack, Mr. Arthur Coquelin and Mr. Robert Sawchuk for their technical assistance.

REFERENCES

1. Grisham, J.W. (1962) Cancer Res. 22, 842-849.
2. Lieberman, I. and Kane, P. (1965) J. Biol. Chem. 240, 1737-1741.
3. Scornik O.A. (1974) J. Biol. Chem. 249, 3876-3883.
4. Rizzo, A.J. and Webb, T.E. (1972) Eur. J. Biochem. 27, 136-144.
5. Zweig, M. and Grisham, J.W. (1971) Biochim. Biophys. Acta 246, 70-80.
6. Von der Decken, A. and Hultin, T. (1958) Exptl. Cell Research 14, 88-96.
7. Tsukada, K. and Lieberman, I. (1965) Biochim. Biophys. Res. Commun. 19, 702-707.
8. Majumdar, C., Tsukada, K. and Lieberman, I. (1967) J. Biol. Chem. 242, 700-704.
9. Tsukada, K., Moriyama, T., Doi, O. and Lieberman, I. (1968) J. Biol. Chem. 243, 1152-1159.
10. Chandler, A.M. and Snider, G.A. (1970) Proc Soc. Exp. Biol. Med. 135, 415-418.
11. Skogerson, L. and Moldave, K. (1967) Biochem. Biophys. Res. Commun. 27, 568-572.
12. Martin, T.E. and Wool, I.G. (1968) Proc Natl. Acad. Sci. U.S.A. 60, 569-574.
13. Gasior, E. and Moldave, K. (1970) J. Mol. Biol. 66, 391-402.
14. Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186-202.
15. Moldave, K., Galasinski, W. and Rao, P. (1971) in Methods in Enzymology XX, K. Moldave and L. Grossman, eds., Academic Press, N.Y., p. 337-348.
16. Siler, J. and Moldave, K. (1969) Biochim. Biophys. Acta 195, 123-129.
17. Moldave, K. (1963) in Methods in Enzymology VI, S.P. Colowick and N.O. Kaplan, eds., Academic Press, N.Y., p. 757-761.
18. Hardy S.J.S., Kurland, C.G., Voynow, P. and Mora, G. (1969) Biochemistry 8, 2897-2905.
19. McFarlane, A.S. (1958) Nature (London) 182, 53.
20. Miller, R.V. and Sypherd, P.S. (1973) J. Mol. Biol. 78, 527-538.
21. Kaltschmidt, E. and Wittmann, H.G. (1970) Anal. Biochem. 36, 401-412.
22. Moss, B.M. and Ingram, V.M. (1965) Proc. Natl. Acad. Sci. U.S.A. 54, 967-974.
23. Sherton, C.C. and Wool, I.G. (1972) J. Biol. Chem. 247, 4460-4467.
24. Ventimiglia, F.A. and Wool, I.G. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 350-354.
25. Eil, C. and Wool, I.G. (1973) J. Biol. Chem. 248, 5122-5129.
26. Ibuki, F. and Moldave, K. (1968) J. Biol. Chem. 243, 791-798.
27. Terhost, C.P., Moller, W., Laursen, R. and Wittman-Liebold, B. (1973) Eur. J. Biochem. 34, 138-152.
28. Kischa, K., Miller, W. and Stoffler, G. (1971) Nature 233, 62-63.
29. Hammel, E. and Nakamoto, T. (1972) J. Biol. Chem. 247, 805-814.
30. Kung, H., Fox, J.E., Spears, C., Brot, N. and Weissbach, H. (1973) J. Biol. Chem. 248, 5012-5015.
31. Ramagopal, S. and Subramanian, A.R. (1974) Proc Natl. Acad. Sci. U.S.A. 71, 2136-2140.