Proceedings of the National Academy of Sciences
Vol. 67, No. 2, pp. 644–651, October 1970

Isolation of RNA with Properties of Messenger RNA from Cerebral Polyribosomes*

Claire E. Zomzely†, Sidney Roberts, and Susan Peache

DEPARTMENT OF BIOLOGICAL CHEMISTRY, SCHOOL OF MEDICINE, AND THE BRAIN RESEARCH INSTITUTE, UNIVERSITY OF CALIFORNIA CENTER FOR THE HEALTH SCIENCES, LOS ANGELES, CALIFORNIA 90024

Communicated by H. W. Magoun, July 24, 1970

Abstract. RNA which dissociated from purified cerebral polyribosomes of adult rats in the presence of EDTA was isolated by fractionation in a discontinuous sucrose gradient. The yield was 2% of the total polyribosomal RNA. The base composition resembled the complementary values for rat DNA and was very different from base compositions of ribosomal RNA and transfer RNA. This RNA fraction contained a large proportion of molecules which were rapidly labeled *in vivo* and hybridized to homologous DNA. The polyribosomal RNA preparation also exhibited high template activity in a cerebral cell-free system which had previously been stripped of the capacity to incorporate amino acids in the absence of added messenger RNA (mRNA). Sedimentation analysis revealed only two peaks, with coefficients of approximately 8 S and 16 S. The data indicate that RNA with the properties of mRNA can be selectively isolated from cerebral polyribosomes under mild conditions which avoid degradation.

Numerous studies support the concept that alterations in RNA and protein synthesis are associated with information transfer and storage in the central nervous system. Activation of the synthesis of specific proteins is suggested by the reports that new RNA with altered base composition, presumably mRNA, is formed during the acquisition phase of learning.[1–4] These findings indicate that successful isolation of undegraded mRNA from brain may lead to definitive studies on the relationship between protein synthesis and the specialized functions of this organ.

Previous investigations from this laboratory revealed that cerebral cortex of the adult rat contains a high proportion of mRNA–ribosome complexes which readily dissociate in media of low Mg^{2+} concentration.[5,6] The same studies indicated that purified cerebral polyribosomes were devoid of measurable RNase activities. These results suggested that mRNA species present in unstable cerebral polyribosomes might be readily extracted in their native state. The present report provides experimental support for this prospect.

Materials and Methods. Polyribosomes, pH 5 fraction and ribosomal factors: Polyribosomes were isolated from cerebral cortical gray matter of young adult male rats (42 days old) without the use of a detergent.[6] About 85% of the total polyribosomes in cerebral postmitochondrial supernatants were recovered in this 'free' polyribosome fraction.[7] The purified polyribosomes contained no detectable RNase activities.[6] The

'pH 5 fractions' were obtained from post-microsomal supernatants of cerebral homogenates by precipitation with 1 N acetic acid at pH 4.7.[8]

Crude ribosomal initiating factors were prepared from cerebral ribosomes by the method of Ghosh, Söll, and Khorana,[9] slightly modified as follows. Cerebral cortical tissue was homogenized in the medium 0.25 M sucrose–10 mM magnesium acetate–60 mM KCl–1 mM dithiothreitol–10 mM Tris·HCl buffer, pH 7.6. Ribosomes were obtained by centrifugation for 2 hr at 40,000 rpm and 0°C and were then resuspended in the same medium to which 1 M NH_4Cl had been added. The suspension was allowed to remain at 0°C for 1 hr prior to removal of the ribosomes by centrifugation. The supernatant fraction was then adjusted to 70% saturation with $(NH_4)_2SO_4$. The resulting precipitate was recovered by centrifugation, dissolved in the medium 1 mM magnesium acetate–1 mM dithiothreitol–10 mM Tris·HCl buffer, pH 7.6, and finally dialyzed against this buffer for 12 hr. The dialyzed protein fraction was the source of the ribosomal factors.

Stripped ribosomes: Ribosomes which contained no detectable RNase activity and were devoid of amino acid-incorporating activity in the absence of added mRNA (stripped ribosomes) were prepared from cerebral mixed ribosomes.[5] The mixed ribosomes were suspended in the medium 0.5 M NH_4Cl–1 mM $MgCl_2$–50 mM Tris·HCl buffer, pH 7.6 and centrifuged for 10 min at 3000 rpm and 0°C. The pellet was discarded. The supernatant was recovered and allowed to remain at 0°C for 1 hr prior to recentrifugation. The resulting supernatant (4 ml) was then layered onto a sucrose density gradient composed of successive 4-ml layers of 0.5 and 1.0 M sucrose, each containing the same buffer and salts. The gradient was centrifuged for 6 hr at 50,000 rpm and 0°C. The pellet was rinsed three times in the medium 1 mM $MgCl_2$–50 mM Tris·HCl buffer, pH 7.6. Sedimentation analyses in medium containing 1 mM $MgCl_2$ revealed that this preparation was composed principally of monoribosomes ("80S"). The yield was equivalent to 0.15–0.2 mg ribosomal protein per gram of cortex.

Polyribosomal mRNA preparations: Polyribosomes equivalent to approximately 12 mg of ribosomal protein were gently suspended at 0°C in 8 ml of 0.3 mM EDTA (sodium salt)–0.001% polyvinylsulfate–50 mM KCl–50 mM Tris·HCl buffer, pH 7.6. After 10 min, the suspension was centrifuged for 10 min at 3000 rpm and 0°C. The resulting supernatant was layered onto 8 ml of a 0.5 M sucrose solution which had previously been layered onto 16 ml of a 2.0 M sucrose solution. Both solutions contained the same buffer and other solutes present in the ribosomal suspension. After centrifugation of the gradient for 16.5 hr at 25,000 rpm and 0°C, the top 14 ml was carefully transferred to a flask; 3 N NaCl was added with stirring to a final concentration of 0.1 N NaCl. This was followed by addition of 2.5 vol of cold absolute ethanol. The mixture was stored for 16–18 hr at −20°C and then centrifuged for 30 min at 15,000 rpm and 0°C. The resulting precipitate was dissolved in 2 ml of 0.001% polyvinylsulfate–50 mM Tris·HCl buffer, pH 7.6. This solution was treated with NaCl and ethanol, allowed to remain at −20°C, and centrifuged as described above. The precipitate was subjected to this purification procedure two additional times, then finally redissolved in 50 mM Tris·HCl buffer at pH 7.6, recovered by centrifugation, and dried under reduced pressure for storage at −60°C. RNA content of this polyribosomal mRNA preparation was measured colorimetrically.[10]

The physical state of the ribosomes after removal of the mRNA fraction was also determined. The residual gradient was made up to about 1 M sucrose and centrifuged for 18 hr at 40,000 rpm and 0°C. The pellet was immediately subjected to sedimentation analysis.

Ribosomal RNA and transfer RNA: Purified rRNA was prepared from cerebral polyribosomes by the extraction procedure of Schneider and Roberts.[11] Transfer RNA was isolated as described by Samli and Roberts[12] from the pH 5 fraction of the postmicrosomal supernatant from cerebral cortex.[8]

Sucrose density gradient analyses: Sedimentation analyses were carried out in linear sucrose gradients (5–20%) at 0–4°C.[6] Stripped ribosomes were suspended in 1 mM $MgCl_2$–25 mM KCl–50 mM Tris·HCl, pH 7.6. Ribosomes remaining after isola-

tion of the mRNA fraction from polyribosomes were suspended in 0.3 mM EDTA (sodium salt)–0.001% polyvinylsulfate–50 mM KCl–50 mM Tris·HCl, pH 7.6. These ribosomal suspensions contained 0.5 mg of protein in 2 ml. Polyribosomal mRNA (0.3 mg) was suspended in 2 ml of 3 mM EDTA (sodium salt)–10 mM NaCl–10 mM sodium acetate buffer, pH 5.2. Suspensions were layered directly onto 28-ml gradients which contained buffer and salts in the same concentrations as the suspending media. After centrifugation of the gradients in an SW 25.1 rotor, 40% sucrose was slowly injected through a puncture hole at the bottom of the plastic centrifuge tube and ultraviolet absorbances of the effluent were recorded continuously at 254 nm.[5]

Base analyses: Base composition of the mRNA preparation from cerebral polyribosomes was determined essentially by the method of Katz and Comb[13] as described by Bondy and Roberts.[14]

RNA·DNA hybridization: Radioactive mRNA and rRNA fractions for hybridization studies were prepared from cerebral polyribosomes of rats given an intracisternal injection of 1 mCi of [5-^{3}H]uridine in 0.9% NaCl (sp act 4 Ci/mol; Schwarz Bioresearch). DNA was isolated from cerebral cortical tissue by the method of Marmur.[15] The capacity of the radioactive RNA preparations to hybridize with denatured DNA retained on nitrocellulose filters was measured as described by Bondy and Roberts.[16] All assays were performed in triplicate.

Template activity of RNA preparations: Stripped ribosomes were suspended for incubation in medium composed of 50 mM Tris·HCl, pH 7.6, and appropriate concentrations of salts. Aliquots were taken for estimation of ribosomal protein.[17] The medium contained (in 1 ml): 50 mM Tris·HCl (pH 7.6), 100 mM KCl, 7 or 12 mM $MgCl_2$, 2 mM ATP (sodium salt), 0.25 mM GTP (sodium salt), 20 mM creatine phosphate (sodium salt), 0.1 mg of creatine phosphokinase, 1 μCi (2.8 nmol) of uniformly labeled [^{14}C]L-phenylalanine (New England Nuclear) or 0.25 μCi of a mixture of uniformly labeled [^{14}C]L-amino acids (New England Nuclear), cerebral stripped ribosomes equivalent to 0.5 mg of protein, 2 mg of pH 5 enzyme protein and, where indicated, ribosomal factors, cerebral RNA preparations, or poly U (Miles Lab).

Incubation was carried out in air at 37°C with occasional agitation. The reaction was stopped by transfer of the tubes to an ice bath and prompt addition of cold trichloroacetic acid containing the appropriate unlabeled amino acids. Precipitation of protein with trichloroacetic acid, purification of the precipitates collected on Millipore filters, and measurement of radioactivity incorporated into protein were carried out as described earlier.[6]

Results. The yield of RNA in polyribosomal mRNA preparations from cerebral cortical gray matter of young adult rats averaged about 2% of the total polyribosomal RNA. The concentration of RNA in these mRNA fractions exceeded 90%; the remainder was largely protein.

Sedimentation properties of cerebral mRNA preparations: Sucrose density gradient analyses of the mRNA preparations from cerebral polyribosomes revealed two distinct peaks with sedimentation coefficients of about 8 S and 16 S (Fig. 1*a*). Removal of this RNA was accompanied by complete dissociation of the ribosomes to their subunits, but did not result in detectable degradation. Sedimentation analysis of the dissociated ribosomes revealed only two peaks with sedimentation coefficients of about 36 S and 26 S (Fig. 1*b*). These two species were present in a mass ratio of 2:1 and were converted to a single peak with the sedimentation value of monomeric ribosomes (80 S) in the presence of 4 mM $MgCl_2$. The 36S and 26S components seem to correspond to "60S" and "40S" ribosomal subunits with sedimentation values reduced by conformational alterations in the presence of EDTA.[5] RNA present in the original polyribosomes was quantitatively recovered in the ribosomal subunits and mRNA fraction.

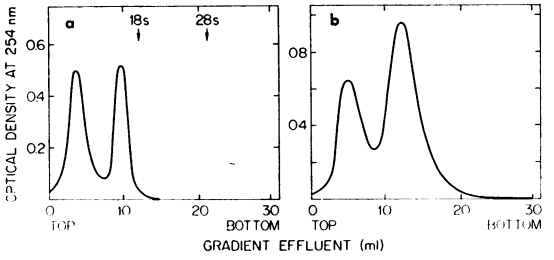

FIG. 1. (*a*): Sedimentation profile of the polyribosomal mRNA preparation from cerebral cortex of the adult rat. The sucrose gradient was centrifuged for 18 hr at 25,000 rpm. The arrows at "18 S" and "28 S" mark the positions of the two major peaks of cerebral rRNA analyzed under similar conditions.

(*b*): Sedimentation profile of ribosomes recovered after removal of the mRNA fraction from cerebral polyribosomes. The sucrose gradient was centrifuged for 16 hr at 18,000 rpm.

Base composition of cerebral mRNA preparations: Polyribosomal mRNA fractions from rat cerebral cortex exhibited base ratios (G + C)/(A + U) which averaged 0.87 (Table 1). Similar values have been reported for the base ratios of hybridizable RNA in crude RNA preparations from rat brain.[16,18,19] The complementary base ratio (G + C)/(A + T) of 0.75 has been obtained for rat liver DNA.[20] The mRNA fraction from cerebral polyribosomes was not grossly contaminated with rRNA, tRNA, or their breakdown products. Thus, analyses of the base compositions of various rRNA preparations from adult rat brain have given ratios of 1.51–1.92.[14,18,19,21] Moreover, the corresponding values for fractionated 28S and 18S rRNA components from cerebral cortex of adult rats were 1.82 and 1.34, respectively.[11] Finally, the (G + C)/(A + U) ratio for the tRNA fraction from rat cerebral cortex exceeded 1.5.[21]

Hybridization of cerebral RNA fractions with DNA: The capacity of mRNA preparations from cerebral polyribosomes to hybridize with homologous DNA varied from 6.4 to 8.8% of the total radioactivity in the RNA within 2 hr after administration of radioactive uridine. No attempt was made to determine the conditions for maximum labeling of the hybridizable material. However, the hybridization capacity of cerebral rRNA, 2 days after the injection of the radio-

TABLE 1. *Base composition of cerebral RNA fractions from adult rats.*

	Base composition (%)				G + C
	C	A	G	U (or T)	A + U (or T)
Polyribosomal mRNA	19.1 ± 0.88	23.3 ± 1.28	27.3 ± 0.98	30.4 ± 1.30	0.87 ± 0.06 (6)
Ribosomal RNA[14]	27.6	17.0	32.4	21.5	1.57 (2)
Hybridizable nuclear RNA[16]	21.7 ± 1.7	21.7 ± 0.9	28.3 ± 1.4	28.3 ± 0.9	1.00 ± 0.03 (4)
Rat liver DNA[20]	21.6	28.8	21.5	28.5	0.75

The values are expressed as means ± SE (where indicated) with the number of analyses in parentheses.

active precursor, was only about one-tenth the values for the mRNA fraction (i.e., 0.74–0.80%). Uniform labeling of the two major rRNA species present has occurred after this time interval (Schneider and Roberts, unpublished observations).

Template activity of cerebral mRNA preparations: Cerebral stripped ribosomes were inactive in amino acid incorporation in an otherwise complete system which included the pH 5 fraction from cerebral cortical tissue (Fig. 2*a*).

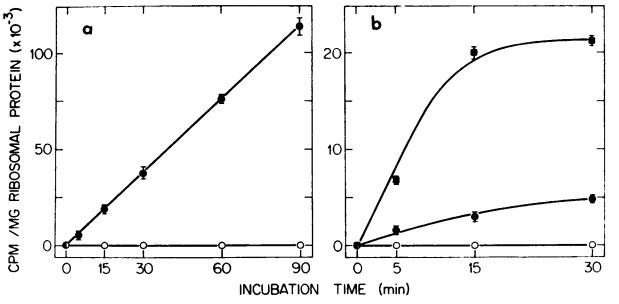

FIG. 2. (*a*): Kinetics of incorporation of [^{14}C]L-phenylalanine by cerebral stripped ribosomes in the presence and in the absence of poly U. The Mg^{2+} content of the incubation medium was 12 mM. Each value represents the mean ± SE (where indicated) for three determinations. O, without poly U; ●, with poly U (600 μg per mg ribosomal protein).

(*b*): Kinetics of incorporation of amino acids into protein of cerebral stripped ribosomes in the presence and in the absence of the polyribosomal mRNA preparation. The incubation mixture contained a mixture of 15 [^{14}C]L-amino acids; the Mg^{2+} content was 7 mM. Each value represents the mean ± SE (where indicated) for 3 determinations. O, without the mRNA fraction; ●, with 150 μg of the mRNA fraction; ■, with 150 μg of the mRNA fraction +60 μg of ribosomal factor protein.

When saturating amounts of the synthetic messenger poly U were added, polyphenylalanine synthesis proceeded at a rapid and linear rate for at least 90 min. The cerebral mRNA fraction also elicited the incorporation of radioactivity into protein when either [^{14}C]L-phenylalanine (Table 2) or a mixture of 15 [^{14}C]L-amino acids (Fig. 2*b*) was incubated with cerebral stripped ribosomes in the presence of the pH 5 fraction. The stripped ribosomes appeared to be deficient in the initiator proteins described in other cell systems.[22–25] When crude ribosomal factors were added to the cerebral system which contained the mRNA preparation, incorporation of amino acids was markedly stimulated over values observed in their absence. Ribosomal factor protein was without effect in the absence of the mRNA preparation. The capacity of cerebral RNA to elicit incorporation of amino acids into protein in the presence of cerebral stripped ribosomes appeared to be specific for the polyribosomal mRNA fraction. Cerebral rRNA (200 μg), as well as cerebral tRNA in high concentration (400 μg), were completely ineffective in activating amino acid incorporation in this system.

Discussion. Considerable progress has been made in the isolation of mRNA for hemoglobin synthesis from polyribosomes of mammalian reticulocytes.[26–31] However, prior efforts to obtain mRNA from polyribosomes of other mammalian cells have been limited by the difficulty of removing mRNA from ribosomal at-

TABLE 2. *Stimulation of incorporation of [^{14}C]L-phenylalanine into protein of cerebral stripped ribosomes in the presence of the polyribosomal mRNA preparation.*

Polyribosomal mRNA	pH 5 fraction	Ribosomal factors	Amino acid incorporation (cpm/mg ribosomal protein)
−	−	−	0
−	+	+	0
+	+	−	5,280 ± 360
+	+	+	23,400 ± 1,150

The Mg^{2+} content of the incubation medium was 12 mM. Amounts of added materials were as follows: polyribosomal mRNA, 110 μg; ribosomal factor protein, 120 μg in the absence of the mRNA preparation and 60 μg in its presence. Incubation was carried out for 20 min at 37°C. Each incorporation value is the mean ± SE (where indicated) for three determinations.

tachment and by degradation of the detached mRNA by RNase. These problems have been avoided in the present investigations by taking advantage of certain unique properties of cerebral polyribosomes from adult rats. These polyribosomes occur mainly unbound or loosely bound to membranes,[7] contain little or no RNase contamination when purified by zone centrifugation in high-density sucrose solutions,[6] and dissociate readily at low concentrations of Mg^{2+}.[5] Dissociation of cerebral polyribosomes under these conditions appears to result in the release of mRNA in a largely undegraded form.

Earlier studies of brain mRNA have dealt with crude nuclear, cytoplasmic, or total RNA fractions which were grossly contaminated with other RNA species.[12,14,16,18,19,32–36] The base composition of polyribosomal mRNA preparations in the present studies revealed that contamination with other nucleic acid species was minor. These base values also indicated a high degree of complementarity with homologous DNA, even though only highly selected base sequences in DNA appear to be expressed in the mRNA produced by any cell of highly-differentiated tissues.[37] Considerable complementarity of base sequences between the polyribosomal mRNA preparation and rat brain DNA was further demonstrated by the relatively high capacity of these two materials to hybridize.

Rapidly-labeled RNA fractions extracted from polyribosomes of a variety of mammalian tissues have often revealed considerable heterogeneity in sucrose density gradients.[38] However, under conditions which reduce degradation, these components appear in more restricted regions of the gradient.[39–42] Polyribosomal mRNA fractions isolated from adult rat brain by the present method revealed only two major peaks sedimenting at 8 S and 16 S. Nuclear RNA preparations from rat brain also contain species with apparent template activity which sediment at approximately 8 S[12] and 16 S.[14,32] Species of mRNA with these sedimentation coefficients would presumably code for proteins with molecular weights approximating 16,000 and 65,000 respectively.[43]

The definitive criterion for identifying natural mRNA is the capacity to induce *de novo* the synthesis of a specific polypeptide in a cell-free system. This condition has been partially met by mRNA for globin synthesis isolated from rabbit reticulocytes.[31] In lieu of this property, the capacity of RNA preparations to stimulate amino acid incorporation into proteins of ribosomal systems has often been measured. However, RNA other than mRNA may have stimula-

tory activity *per se* or may activate a ribosomal system which contains endogenous mRNA.[44-47] In the present investigations, this problem was minimized by assaying RNA template activity in the presence of cerebral ribosomes which had previously been stripped of endogenous mRNA, as well as other factors required for protein synthesis. These "stripped" ribosomes were completely inactive without added mRNA in an otherwise complete amino acid-incorporating system which included ribosomal initiating factors and pH 5 enzymes. Addition of the polyribosomal mRNA preparation resulted in highly active incorporation of amino acids into protein. The nature of proteins which may be synthesized under these conditions and their relationship to cerebral function remain to be elucidated.

* This work was supported by research grants from the National Institutes of Health (NS-07869).

† Present address: Roche Institute for Molecular Biology, Nutley, N. J. 07110.

[1] Hydén, H., and E. Egyházi, *Proc. Nat. Acad. Sci. USA*, **49**, 618 (1963).
[2] Hydén, H., and P. W. Lange, *Proc. Nat. Acad. Sci. USA*, **53**, 946 (1965).
[3] Shashoua, V. E., *Nature*, **217**, 238 (1968).
[4] Shashoua, V. E., *Proc. Nat. Acad. Sci. USA*, **65**, 160 (1970).
[5] Zomzely, C. E., S. Roberts, D. M. Brown, and C. Provost, *J. Mol. Biol.*, **20**, 455 (1966).
[6] Zomzely, C. E., S. Roberts, C. P. Gruber, and D. M. Brown, *J. Biol. Chem.*, **243**, 5396 (1968).
[7] Roberts, S., C. E. Zomzely, and S. C. Bondy, in *Cellular Aspects of Growth and Differentiation in Nervous Tissue*, ed. D. C. Pease (UCLA Forum in Medical Sciences), in press.
[8] Zomzely, C. E., S. Roberts, and D. Rapaport, *J. Neurochem.*, **11**, 567 (1964).
[9] Ghosh, H. P., D. Söll, and H. G. Khorana, *J. Mol. Biol.*, **25**, 275 (1967).
[10] Webb, J. M., *J. Biol. Chem.*, **221**, 635 (1956).
[11] Schneider, D., and S. Roberts, *J. Neurochem.*, **15**, 1469 (1968).
[12] Samli, M. H., and S. Roberts, *J. Neurochem.*, **16**, 1565 (1969).
[13] Katz, S., and D. G. Comb, *J. Biol. Chem.*, **238**, 3065 (1963).
[14] Bondy, S. C., and S. Roberts, *Biochem. J.*, **105**, 1111 (1967).
[15] Marmur, J., *J. Mol. Biol.*, **3**, 208 (1961).
[16] Bondy, S. C., and S. Roberts, *Biochem. J.*, **109**, 533 (1968).
[17] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[18] Jacob, M., J. Stevenin, R. Jund, C. Judes, and P. Mandel, *J. Neurochem.*, **13**, 619 (1966).
[19] Stevenin, J., J. Samec, M. Jacob, and P. Mandel, *J. Mol. Biol.*, **33**, 777 (1968).
[20] Wyatt, G. R., *Biochem. J.*, **48**, 584 (1951).
[21] Mahler, H. R., W. J. Moore, and R. J. Thompson, *J. Biol. Chem.*, **241**, 1283 (1966).
[22] Iwasaki, K., S. Sabol, A. J. Wahba, and S. Ochoa, *Arch. Biochem. Biophys.*, **125**, 542 (1968).
[23] Miller, R. L., and R. Schweet, *Arch. Biochem. Biophys.*, **125**, 632 (1968).
[24] Revel, M., J. C. Lelong, G. Brawerman, and F. Gros, *Nature*, **219**, 1016 (1968).
[25] Fuhr, J. E., I. M. London, and A. I. Grayzel, *Proc. Nat. Acad. Sci. USA*, **63**, 129 (1969).
[26] Chantrenne, H., A. Burny, and G. Marbaix, in *Progress in Nucleic Acid Research and Molecular Biology*, ed. J. N. Davidson and W. E. Cohn (New York: Academic Press, 1967), vol. 7, p. 173.
[27] Lanyon, W. G., J. Paul, and R. Williamson, *FEBS (Fed. Eur. Biochem. Sci.) Letters*, **1**, 279 (1968).
[28] Noll, H., and E. Stutz, in *Methods in Enzymology*, ed. L. Grossman and K. Moldave (New York: Academic Press, 1968), vol. XII, Part B, p. 129.
[29] Hunt, J. A., and D. G. Laycock, in *Cold Spring Harbor Symp. Quant. Biol.*, **34**, 579 (1969).
[30] Labrie, F., *Nature*, **221**, 1217 (1969).
[31] Laycock, D. G., and J. A. Hunt, *Nature*, **221**, 1118 (1969).
[32] Herriman, I. D., and G. D. Hunter, *J. Neurochem.*, **12**, 937 (1965).
[33] Egyházi, E., and H. Hydén, *Life Sci.*, **5**, 1215 (1966).
[34] Yamagami, S., R. R. Fritz, and D. A. Rappoport, *Biochim. Biophys. Acta*, **129**, 532 (1966).

[35] Kimberlin, R. H., *J. Neurochem.*, **14**, 123 (1967).
[36] Vesco, C., and A. Giuditta, *Biochim. Biophys. Acta*, **142**, 385 (1967).
[37] Bolton, E. T., and B. J. McCarthy, *J. Mol. Biol.*, **8**, 201 (1964).
[38] Hadjiolov, A. A., in *Progress in Nucleic Acid Research and Molecular Biology*, ed. J. N. Davidson and W. E. Cohn (New York: Academic Press, 1967), vol. 7, p. 195.
[39] Henshaw, E. C., M. Revel, and H. H. Hiatt, *J. Mol. Biol.*, **14**, 241 (1965).
[40] Hadjiolov, A. A., *Biochim. Biophys. Acta*, **119**, 547 (1966).
[41] Kuff, E. L., and W. C. Hymer, *Biochemistry*, **5**, 959 (1966).
[42] Tsanev, R. G., G. G. Markov, and G. N. Dessev, *Biochem. J.*, **100**, 204 (1966).
[43] Kurland, C. G., *J. Mol. Biol.*, **2**, 83 (1960).
[44] Nirenberg, M. W., and J. H. Matthaei, *Proc. Nat. Acad. Sci. USA*, **47**, 1588 (1961).
[45] Maxwell, E. S., *Proc. Nat. Acad. Sci. USA*, **48**, 1639 (1962).
[46] Kruh, J., J. C. Dreyfus, and G. Schapira, *Biochim. Biophys. Acta*, **91**, 494 (1964).
[47] Shaeffer, J., G. Favelukes, and R. Schweet, *Biochim. Biophys. Acta*, **80**, 247 (1964).