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Differential Compartmentalization of Messenger Ribonucleic Acid in Murine Testis†

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ABSTRACT: Messenger ribonucleic acid (mRNA) from fractionated mouse testis has been used as a template in the wheat germ and reticulocyte lysate cell-free translation systems. Cell-free translation products of deproteinized RNA from testis polysomes and from a nonpolysomal fraction (< 80 S) have been compared by one- and two-dimensional polyacrylamide gel electrophoresis, followed by autoradiography. Wheat germ and reticulocyte ribosomes translate both polysomal and nonpolysomal RNA from testis with high efficiency. Analysis of the polypeptide products of these cell-free translation systems indicates a compartmentalization between polysome-bound and nonpolysomal mRNA in testis. With the assumption of equal template efficiency for those RNAs tested, three classes of radiolabeled polypeptide products have been distinguished: (1) polypeptide bands which represent an equal abundance of mRNA in each cell compartment; (2) polypeptide bands which represent a higher abundance of mRNA in the polysomal than in the nonpolysomal compartment; (3) polypeptide bands which represent a higher abundance of mRNA in the nonpolysomal than in the polysomal compartment. Compared with liver, the testis contained a larger proportion of ribosomes present as monosomes. Further, more poly(A)+ RNA and an equal or greater template activity were found to be associated with the nonpolysomal portion of testis cytoplasm than the polysomal fraction, suggesting that testis does not have as large a proportion of its messenger RNAs actively involved in protein synthesis as does liver. A comparison of cell-free translation products of deproteinized and nondeproteinized RNAs obtained from total testis cytoplasm had revealed similar polypeptide profiles with a few minor differences. These data suggest that some form of selective mRNA masking or sequestration in a subcellular compartment may be regulating the loading of specific nonpolysomal mRNAs onto polysomes.

Evidence for the control of gene expression at the level of selective translation has begun to accumulate in recent years. Investigation of the development of sea urchin oocytes (Davidson, 1976), trout testis (Gedamu & Dixon, 1976), and rat testis (Grimes & Kay, 1979), as well as the early embryogenesis of *Spisula* (Rosenthal et al., 1980) and *Xenopus* (Davidson, 1976), have led to the conclusion that nonpolysomal mRNA[1] plays an important role in the regulation of gene expression during development. Further, regulation at this level may be involved in reticulocyte maturation (Jacobs-Lorena & Baglioni, 1972; Civelli et al., 1980) and in Ascites tumor cells (Geoghegan et al., 1978, 1979; McMullen et al., 1979), Friend cells (S. Cereghini, personal communication), and Hela cells (Penman et al., 1968).

Spermatogenesis provides an excellent developmental system in which to study the role of nonpolysomal mRNPs in gene expression. In this well-ordered process, stem cells, spermatogonia, undergo a series of differentiations to produce the highly differentiated spermatozoa. During this prolonged series of events, chromosome pairing and genetic recombination occur during meiotic prophase. This is followed by two divisions yielding haploid spermatids. In an interval of spermatogenesis called spermiogenesis these spermatids will eventually differentiate into spermatozoa. Spermatids are known to be active in protein synthesis (Monesi, 1964; Turkington & Majumder, 1975), but the extent of transcription in the cells is unclear [for review see Monesi et al. (1978)]. Recently, claims have been made that a considerable fraction of ribosomal and poly(A)+ RNA produced by premeiotic and meiotic cells is preserved until late spermiogenesis. Assuming these RNAs play a physiological role in the testis, one might expect that "long-lived" mRNA could be regulated during spermiogenesis by the process of selective translation.

Potential messenger RNA activity can be assayed by translation in the wheat germ cell-free system (Roberts & Paterson, 1973) or in the cell-free reticulocyte lysate system (Pelham & Jackson, 1976). A new method for assessing the messenger template activity of unextracted cell homogenates has been developed (Geoghegan et al., 1979; Rosenthal et al., 1980) to test the regulatory role of phenol-soluble components associated with mRNA. In this report, we compare the polypeptide products derived from cell-free translations of fractionated mouse testis to determine the polypeptides encoded by polysome-bound and nonpolysomal mRNAs. RNA

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[1] Abbreviations used: Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; mRNA, messenger ribonucleic acid; mRNP, messenger ribonucleic acid and associated protein; poly(A), poly(adenylic acid); poly(A)+, containing poly(adenylic acid); poly(A)−, lacking poly(adenylic acid); poly(U), poly(uridylic acid); RNA, ribonucleic acid; RNP, ribonucleic acid and associated proteins; SA, specific activity; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; PPO 2,5-diphenyloxazole; Me_2SO, dimethyl sulfoxide.

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isolated from subcellular fractions of mouse liver provides a useful comparison tissue for these studies.

Experimental Procedures

Cell Fractionation. Sexually mature CD-1 male mice at least 45 days old were obtained from Charles River Breeding Laboratories, North Wilmington, MA. After sacrifice by cervical dislocation, the testis was dissected, and the tunica albuginea was removed with forceps. The remainder of the testis was then washed 3 times at 4 °C in a wash buffer (50 mM Tris-HCl, pH 7.6, 100 mM KCl, and 1.5 mM $MgCl_2$) at 2 mL/g of tissue. The washed, decapsulated testis was then resuspended in the wash buffer containing 0.1% Triton X-100 v/v (Sigma, St. Louis, MO) and 0.1% diethyl pyrocarbonate v/v (Sigma, St. Louis, MO). The testis was then homogenized with a motor-driven Teflon pestle for seven strokes in a glass homogenizer. Unbroken cells, cell debris, and nuclei were pelleted and discarded after a 10-min centrifugation at 1500*g*. Mitochondria were pelleted and discarded after a 10-min centrifugation of the supernatant at 12000*g*. To fractionate the postmitochondrial supernatant into polysomal and nonpolysomal components, we used sucrose gradient centrifugation.

Sucrose Gradients. Postmitochondrial supernatants of whole testis homogenates were loaded onto linear 10–60% sucrose w/v (Scwarz/Mann, Orangeburg, NY) gradients containing 50 mM Tris-HCl, pH 7.6, 50 mM KCl, and 1.5 mM $MgCl_2$ and centrifuged in an SW-27 rotor (Spinco Division, Beckman Instruments, Inc., Palo Alto, CA) at 25000 rpm for 2.5 h at 4 °C to recover polysomes *or* 19 h at 4 °C to recover testis RNPs < 80 S (hereafter called the nonpolysomal fraction). We define the latter as the fraction of testis RNP which sediments more slowly than the monosome peak in 19-h sucrose gradients. Under these longer centrifugation conditions most polysomes pelleted. Following centrifugation, gradients were fractionated by puncturing the bottom of the cellulose nitrate tubes and pumping them out with 2 M sucrose through an Altex UV monitor to determine absorbance at 260 nm (Altex Division of Beckman Instruments, Inc., Berkeley, CA). Nonpolysomal and polysomal fractions obtained from sucrose gradients were then precipitated by the addition of sodium acetate to a final concentration of 2% and 2.5 volumes of absolute ethanol and stored overnight at −20 °C.

Phenol Extraction of RNA. The precipitated polysomal or nonpolysomal fractions from sucrose gradients or from the postmitochondrial supernatants of tissues were pelleted by centrifugation at 4 °C for 10 min at 10000*g*. The pellets were then resuspended in 100 mM Tris-HCl, pH 9.0, 0.5% $NaDodSO_4$ w/v (Sigma, St. Louis, MO), and 0.1% diethyl pyrocarbonate v/v, and an equal volume of cold, freshly distilled, water-saturated phenol was added. This mixture was throughly vortexed and then centrifuged to separate phases. After reextraction of both the first phenol and first aqueous phases, the aqueous phases were pooled and reextracted with an equal volume of mixed phenol/chloroform/isoamyl alcohol (48:48:4). The aqueous phase was then reextracted with the organic mixture until a clear interface was observed. RNA was precipitated as described above.

Total Testis Cytoplasmic Pellets. Testes were decapsulated and homogenized in 50 mM Tris-HCl, pH 7.6, 100 mM KCl, and 1.5 mM $MgCl_2$ as above. Postmitochondrial supernatants obtained as described previously were centrifuged for 3.5 h at 50000 rpm in a Type 65 rotor (Spinco Division, Beckman Instruments, Inc., Palo Alto, CA). The pelleted fractions from the total cytoplasmic supernatants were then resuspended in 20 mM Hepes, pH 7.5 (Sigma Chemical Co, St. Louis, MO) containing 120 mM KCl and 1.5 mM $MgCl_2$ in preparation for translation in a heterologous reticulocyte cell-free system (Geoghegan et al., 1979; Rosenthal et al., 1980).

Protein Synthesis in Wheat Germ Extracts. The reaction mixtures (total 25 μL) contained 5 μL of wheat germ extract (Roberts & Paterson, 1973), 24 mM Hepes, pH 7.2, 0.8 mM spermidine, 1 mM magnesium acetate, 2 mM dithiothreitol, 1.2 mM 2-mercaptoethanol, 1 mM ATP, 0.2 mM GTP, 8 mM creatine phosphate, 116 mM potassium acetate, 24 mM KCl, 50 μg/mL creatine phosphokinase, 30 μM each of all the common amino acids except methionine, 10 μCi of [^{35}S]-methionine (translation grade, New England Nuclear, Boston, MA; SA = 1200 Ci/mmol), and variable amounts of exogenous RNA. After incubation at 22 °C for 2 h, pancreatic ribonuclease A (Sigma, St. Louis, MO) was added to a final concentration of 200 μg/mL, and the reaction mixture was digested at 37 °C for 30 min. Aliquots of 2.5 μL were removed for measurement of [^{35}S]methionine incorporated into the hot trichloroacetic acid insoluble fraction.

Protein Synthesis in Reticulocyte Lysates. Reticulocyte lysate, preincubated with micrococcal nuclease (10 μg/mL) for 10 min at 20 °C and supplemented with all the common amino acids minus methionine, was obtained from Amersham Corp. (Arlington Heights, IL). The reaction mixture (total 25 μL) consisted of 16 μL of lysate, 5 μL of exogenous RNA or RNP, and 20 μCi of [^{35}S]methionine (SA = 1200 Ci/mmol). Reticulocyte lysate cell-free protein synthesis reactions were terminated by incubation of the reaction with pancreatic ribonuclease A (final concentration 200 μg/mL) for 30 min at 37 °C. Incorporation was monitored as described above.

Measurement of Poly(A) Content. Aliquots of deproteinized RNA were annealed to 6 nCi of [^{3}H]poly(U) (20 Ci/mol of P; Miles Laboratories, Kankakee, IL) for 15 min at 25 °C in 0.5 mL of 10 mM Tris-HCl, pH 7.6, 200 mM NaCl, and 5 mM $MgCl_2$. Hybridization to [^{3}H]poly(U) was terminated by an additional 30-min incubation at 25 °C in the presence of 2 μg/mL of pancreatic ribonuclease A. The remaining [^{3}H]poly(U) was precipitated with cold 10% trichloroacetic acid.

Polyacrylamide Gel Electrophoresis and Autoradiography. Electrophoresis was performed on 13% polyacrylamide slab gels (0.8 mm thick) containing 0.1% $NaDodSO_4$ and 5.5 M urea, with a 5.5% polyacrylamide stacking gel containing 2.7 M urea (Storti & Rich, 1976). Equal volumes of labeled "in vitro" reaction mixtures were applied to the gel after adjusting to 50 mM Tris-HCl, pH 6.8, 2% $NaDodSO_4$, 35 mM 2-mercaptoethanol, and 10% glycerol. Electrophoresis was carried out at 200 V for 24 h. The gels were stained with Coomassie brilliant blue R (Sigma, St. Louis, MO), destained, and dried onto 3-MM Whatman paper "in vacuo". Autoradiograms of [^{35}S]methionine-labeled samples were obtained by exposure to Kodak X-O-Mat R film (Eastman-Kodak, Rochester, NY) at room temperature.

Isoelectric Focusing. Isoelectric focusing in disc gels was carried out according to the procedure of O'Farrell (1975). Equal volumes of cell-free protein synthesis reactions were dissolved in a sample buffer containing 9.5 M urea, 2% NP-40 v/v, 2% ampholytes v/v, and 5% 2-mercaptoethanol v/v. These samples (100 μL) were loaded onto 12 × 0.2 cm cylindrical gels which were electrofocused for 6500 V-h. The focused gels were extruded from the glass tubes and equilibrated in an $NaDodSO_4$-containing sample buffer (Laemmli, 1970) for 2 h. For determination of the pH gradient established, the pH of electrofocused gels containing no protein was determined from 12 equal pieces, each of which was suspended

Table I: Distribution of Total and Poly(A)+ RNA in Polysomal and Nonpolysomal Fractions from Testis and Liver Obtained after Sucrose Gradient Centrifugation

	A[a]		B[b]	
	amount of polysomal RNA[a] (μg of RNA/g of tissue)	amount of nonpolysomal RNA[a] (μg of RNA/g of tissue)	amount of polysomal poly(A)+ RNA[b] (^{3}H cpm bound)	amount of nonpolysomal poly(A)+ RNA[b] (^{3}H cpm bound)
testis	158 ± 12 [7] (53%)	138 ± 21 [5] (47%)	19 000 ± 300 [7] (28%)	49 500 ± 1600 [6] (72%)
liver	154 ± 8 [9] (71%)	64 ± 14 [3] (29%)	5 200 ± 80 [4] (61%)	3 300 ± 160 [3] (39%)

[a] For total RNA measurements, values are expressed as micrograms of RNA recovered in that gradient fraction divided by the wet weight of the tissue in grams ± SEM. The number of different preparations used in making this computation is in brackets; parentheses indicate the percentrage of the total. [b] For measurements of poly(A)$^+$ RNA, aliquots of RNA which had been extracted from shaded sucrose gradient fractions were assayed for poly(A) content with [^{3}H]poly(U). Values are expressed as cpm of [^{3}H]poly(U) which annealed to the total amount of RNA present in each gradient fraction. Numbers are expressed as ^{3}H cpm ± SEM.

in 1 mL of deionized water. Following equilibration, the isoelectric focusing gels were affixed atop an $NaDodSO_4$-urea polyacrylamide slab gel described above.

Fluorography. $NaDodSO_4$-urea polyacrylamide gels were impregnated with PPO-Me_2SO according to the procedure described by Bonner & Laskey (1974). These gels were then washed in cold, running tap water overnight to reduce the radioactive background and dried onto 3-MM Whatman paper in vacuo. Gels were exposed as described for autoradiography except they were stored at −70 °C.

Results

Sedimentation Profiles for Polysomal and Nonpolysomal Fractions from Steady-State Testis and Liver. Preparations of polysomes from testis and liver were obtained following 2.5 h of centrifugation (Figure 1A,C). Since the vast majority of cytoplasmic RNA represents ribosomal RNA, tracings of absorption at 260 nm yield an approximation of the distribution of ribosomes in subcellular fractions. Compared with liver, the testis contained a larger proportion of ribosomes present as monosomes. A longer centrifugation time of 19 h was chosen to isolate RNPs < 80 S from testis and liver in order to optimally separate the polysomal and nonpolysomal components (Figure 1B,D). The shaded areas of Figure 1 reflect the material which was collected as polysomal and nonpolysomal fractions. Fractionation was performed numerous times, with and without detergent, and at ionic strengths in the homogenization buffer up to 200 mM KCl. Similar results were obtained under all these conditions.

Measurement of the absorbance at 260 nm of deproteinized gradient fractions allows for a comparison between them (Table I). The monosome fraction was not collected. The amount of RNA recovered from nonpolysomal fractions of liver gradients is a smaller proportion of the total than obtained in preparations from testis. About half of the RNA recovered from testis is nonpolysomal. This is consistent with the observation that the testis contains a larger proportion of monosomes as compared with liver.

Distribution of Poly(A)+ RNA in Gradient Fractions from Liver and Testis. For determination of the presence of poly(A)+ RNA in the polysomal and nonpolysomal fractions of sucrose gradients, aliquots of RNA obtained from the appropriate gradient fractions were annealed to [^{3}H]poly(U) (Table I). Sixty-one percent of the poly(A)+ RNA in the liver preparations was found to be associated with the polysomal fraction, while thirty-nine percent was found to be associated with the nonpolysomal fraction. The monosomes were not assayed for poly(A)+ RNA. In the testis the situation was reversed. Only 28% of the poly(A)+ RNA was found associated the polysomes, while 72% of the poly(A)+ RNA was found associated with the nonpolysomal fraction of these gradients. These data indicate that more poly(A)+ RNA is present in the nonpolysomal portion of testis than liver.

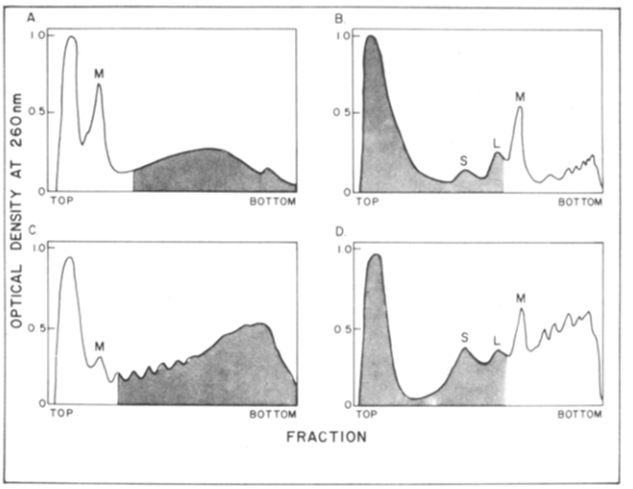

FIGURE 1: Sedimentation profiles of postmitochondrial supernatants from testis and liver. (A) Testis polysome fraction; (B) testis RNP < 80 S fraction; (C) liver polysome fraction; (D) liver RNP < 80 S fraction. (A and C) Sedimentation for 2.5 h. (B and D) Sedimentation for 19 h. M is the monosome peak, L is the large ribosomal subunit peak, and S is the small ribosomal subunit peak. Shaded areas indicate the parts of the gradients collected and extracted as described.

Comparison of Template Activity between Polysomal and Nonpolysomal RNAs. To determine the template activity of RNA in the polysomal and nonpolysomal fractions of sucrose gradients, we assayed aliquots of 4.5 μg of RNA obtained from the appropriate gradient fractions in cell-free translation mixtures (Table II). Adding increasing amounts of exogenous RNA resulted in linearly increasing stimulation of the cell-free systems over a broad range. Further addition resulted in decreased incorporation as seen in Figure 2, lane 6. At the 4.5-μg input level, all incorporations were in the linear part of the incorporation curve. The ^{35}S cpm of trichloroacetic acid insoluble product multiplied by the amount of RNA purified from each shaded gradient fraction gives a relative measure of template activity for polysomal and nonpolysomal RNA (Table II). The vast majority of template-active RNA in liver is found in the polysomes, while a small proportion is found in the nonpolysomal fraction. In mouse testis, however, nonpolysomal RNA contains an equal or larger proportion of the template activity found in polysomal RNA. Further, testis nonpolysomal RNA consistently saturated the wheat germ cell-free system at a lower input level than did the polysomal RNA (data not shown). These observations suggest that testis nonpolysomal RNA contains a higher concentration of poly(A)+ RNA than does polysomal RNA, a conclusion supported by the poly(A) measurement of Table I.

Polypeptides Encoded by Testicular Fractions. $NaDodSO_4$–urea polyacrylamide gel electrophoresis of polypeptides

Table II: Measurement of Cell-Free Protein Synthesis and Total Template Activity of RNA from Polysomal and Nonpolysomal Fractions from Testis and Liver

	wheat germ lysate[a] (^{35}S cpm incorporated/ 2.5-μL aliquot)		reticulocyte lysate[a] (^{35}S cpm incorporated/ 2.5-μL aliquot)		wheat germ lysate[b] (^{35}S cpm incorporated × 10^3/total RNA in fraction)		reticulocyte lysate[b] (^{35}S cpm incorporated × 10^3/total RNA in fraction)	
	polysomal	non-polysomal	polysomal	non-polysomal	polysomal	nonpolysomal	polysomal	nonpolysomal
testis	8 900	11 700	19 400	40 500	14 100 ± 700 (47%)	16 100 ± 2 400 (53%)	30 700 ± 2 300 (36%)	55 700 ± 8 400 (64%)
liver	7 500	2 700	NT[c]	NT	11 600 ± 600 (87%)	1 700 ± 360 (13%)	NT	NT

[a] For measurements of cell-free synthesis, values are expressed as ^{35}S cpm of hot trichloroacetic acid insoluble material in a 2.5-μL aliquot of reaction mixture (total volume = 25 μL). Each of these values represents the amount of radiolabeled protein synthesized by 4.5 μg of the designated RNAs. Endogenous protein synthetic activities were 990 ^{35}S cpm for the wheat germ and 3400 ^{35}S cpm for the reticulocyte lysate. [b] Template activity measurements are expressed as ^{35}S cpm × 10^3 of hot trichloroacetic acetic acid insoluble material synthesized by deproteinized total RNA from each gradient fraction. These numbers were derived by multiplying the cell-free protein synthesis data by the amount of RNA recovered from mouse tissues (Table I). Numbers are expressed as ^{35}S cpm × 10^3 ± SEM. [c] NT, not tested.

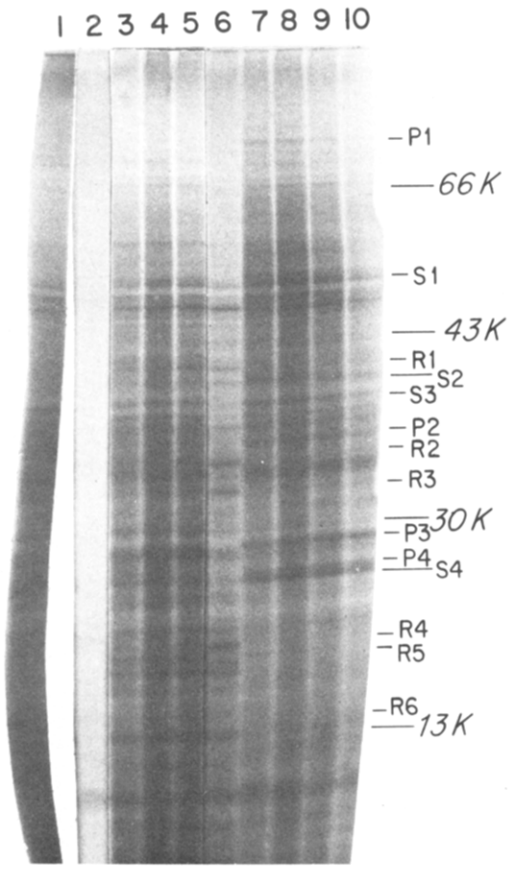

FIGURE 2: Autoradiograph of a one-dimensional polyacrylamide gel analyzing the [^{35}S]methionine-labeled polypeptides from a wheat germ cell-free translation. (Lane 1) Products directed by 4.5 μg of total extracted cytoplasmic RNA. (Lane 2) Products directed by endogenous wheat germ RNA. (Lanes 3–6) Products directed by 1, 2.3, 4.5, and 9 μg, respectively, of testis RNA derived from RNP < 80 S (nonpolysomal RNA). (Lanes 7–10) Products directed by 2.3, 4.5, 9, and 16 μg, respectively, of testis polysomal RNA.

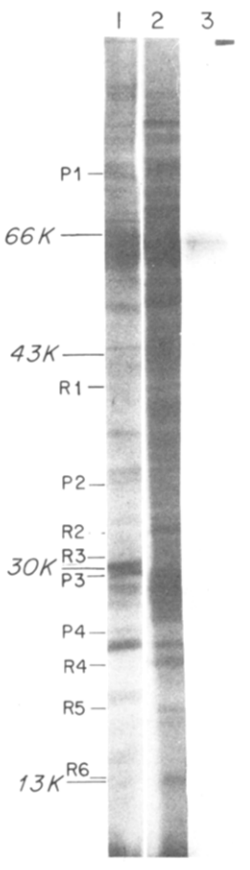

FIGURE 3: Autoradiograph of a one-dimensional polyacrylamide gel analyzing the [^{35}S]methionine-labeled polypeptides from a reticulocyte cell-free translation. (Lane 1) Products directed by 4.5 μg of testis polysomal RNA. (Lane 2) Products directed by 4.5 μg of testis RNA derived from RNP < 80 S. (Lane 3) Products directed by endogenous reticulocyte RNA.

synthesized by the addition of exogenous RNA to the cell-free wheat germ system revealed differences between polysomal and nonpolysomal RNA templates (Figure 2). If equal template efficiency is assumed for the populations of RNA tested, the results can be assigned into three classes. (1) Polypeptide bands such as those denoted as S1–S4 with estimated molecular weights of 50 000, 37 000, 36 000, and 20 000 represent an equal abundance of mRNA in the polysomal and nonpolysomal compartments. (2) Polypeptide bands such as those denoted as P1–P4 with estimated molecular weights of 76 000, 34 000, 26 000, and 21 000 represent a higher abundance of mRNA in the polysomal than in the nonpolysomal portion of the cytoplasm. (3) Polypeptide bands such as those denoted by R1–R6 with estimated molecular weights of 38 000, 32 500, 31 000, 17 000, 16 000, and 14 500 represent a higher abundance of mRNA in the nonpolysomal than in the polysomal fraction (Figure 2). Control experiments in which extracted RNA from total testis cytoplasm was translated revealed bands from all three classes denoted above (Figure 2). The mRNAs encoding the polypeptides of class 3, those which represent a higher abundance of mRNA in the nonpolysomal than in the polysome bound compartment, must therefore be present in substantial amounts in extracts of total cytoplasm.

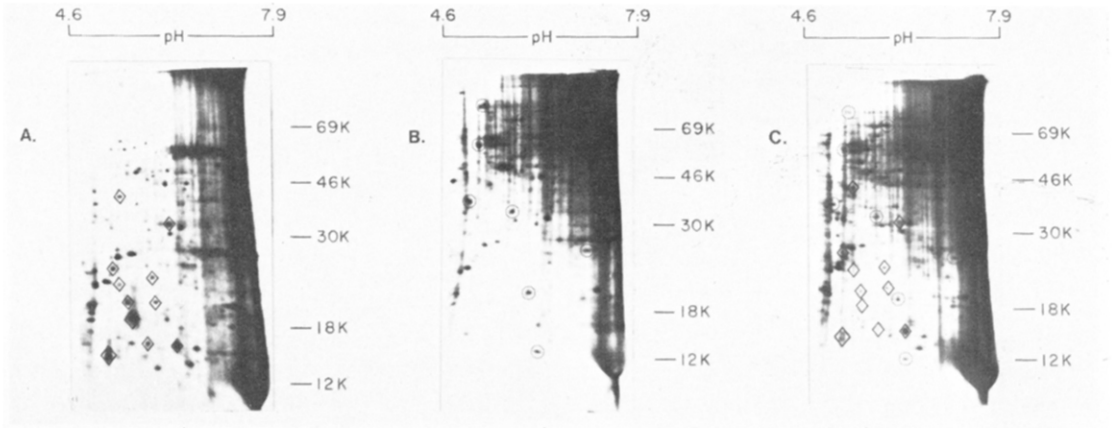

FIGURE 4: Autoradiographs of two-dimensional polyacrylamide gels analyzing the [^{35}S]methionine-labeled polypeptides from a reticulocyte cell-free translation. (A) Products directed by 4.5 μg of testis RNA derived from RNP < 80 S. (B) Products directed by 4.5 μg of testis polysomal RNA. (C) Products directed by 4.5 μg of total extracted cytoplasmic RNA. Diamonds designate spots which represent a higher abundance of mRNA in the nonpolysomal compartment; circles designate spots which represent a higher abundance of mRNA in the polysomal compartment.

A comparison of the polypeptides synthesized in cell-free translations of polysomal and nonpolysomal RNA in reticulocyte lysates substantiates the results obtained with the wheat germ system (Figure 3). In addition, analysis of the polypeptides synthesized by the reticulocyte cell-free system revealed the existence of several high molecular weight polypeptides, encoded by mRNAs present in higher abundance in the polysomal compartment. Several additional high molecular polypeptides appear also in the nonpolysomal translation. Such polypeptides are seen more prominently in translations using reticulocyte lysate than in those using wheat germ extract.

The comparmentalization of many messenger RNAs encoding different polypeptides is best seen when reticulocyte cell-free translations are analyzed by high-resolution two-dimensional gel electrophoresis (Figure 4). The higher proportion of polypeptides >50 000 daltons encoded by mRNA from the polysomal fraction is also apparent (Figure 4B).

Polypeptides Encoded by Extracted and Unextracted Testis Cytoplasmic Pellet RNA. To determine whether proteins or other phenol-soluble factors that bind to mRNA can markedly affect the ability of testicular mRNAs to be translated in a cell-free system, we compared the translation products of deproteinized and nondeproteinized cytoplasmic pellets. Similar profiles were detected when the polypeptides encoded by phenol-extracted RNA were compared to those encoded by unextracted cytoplasm (Figure 5). A few differences, however, such as E1 and E2 can be noted.

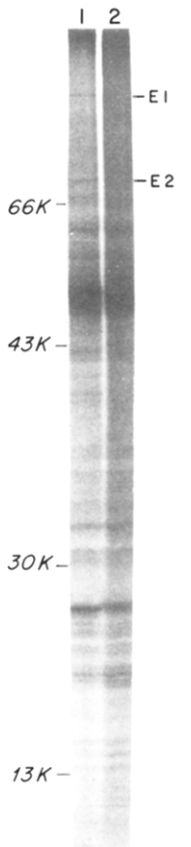

FIGURE 5: Autoradiograph of a polyacrylamide gel analyzing the [^{35}S]methionine-labeled polypeptides from a reticulocyte cell-free translation. (Lane 1) Products directed by 4.5 μg of extracted testis cytoplasmic pellet RNA. (Lane 2) Products directed by equivalent amount of unextracted testis cytoplasmic pellet RNA.

Discussion

Comparisons of RNA present in polysomal and nonpolysomal fractions of mouse testis and liver reveal that in contrast to liver, the testis contains a larger proportion of monosomes and nonpolysomal RNA. (Figure 1, Table I). This suggests that mouse testis has a larger proportion of its protein synthetic apparatus in a "reserved state" than does liver. Moreover, the nonpolysomal fraction of testis cytoplasm contains more poly(A)+ RNA than the polysomal fraction, supporting the idea that the testis has a smaller proportion of its total mRNAs actively involved in protein synthesis than does liver. Although the measurements of poly(A)+ RNA may be influenced by free poly(A) as has been found in some mouse tissues (Bergmann & Brawerman, 1980), the fact that more than half of the template activity found in testis is associated with the nonpolysomal fraction argues against this.

The template activity of liver nonpolysomal RNA is low as compared with liver polysomal RNA. This contrasts with the testis where nonpolysomal RNA has an equal or greater template activity than polysomal RNA. Furthermore, of the proteins encoded by total polysomal and nonpolysomal RNAs from liver, electrophoretic gel analysis indicated no polypeptide band differences. This is in agreement with results found in liver by others (S. Cereghini, personal communication). If random polysomal runoff occurred during our isolation procedure, one would not expect to find differences in testis fractions prepared under identical conditions. Therefore, if polysomal runoff is to account for our results, it must have been selective. We feel the marked differences seen in the proteins encoded by RNAs from polysomal and nonpolysomal

testicular fractions suggest that translational regulation may be utilized to a greater degree during the differentiative processes of spermatogenesis than in the mature liver.

Differences in the distribution of poly(A)+ RNA and template activity between polysomal and nonpolysomal fractions of mouse testis and liver suggest the following (1) RNAs from the nonpolysomal and polysomal fractions of testis both serve as template for cell-free protein synthesis, a conclusion in agreement with that reported for rat testis (Grimes & Kay, 1979). (2) The mouse testis does not have as large a proportion of its RNA actively involved in protein synthesis as does mouse liver. (3) A larger proportion of the poly(A)+ RNA is found associated with nonpolysomal RNA in testis than in liver. (4) Many similar and some different polypeptides are encoded by testis nonpolysomal RNA as compared with polysomal RNA. Similar distributions of proteins encoded by polysomal and nonpolysomal mRNAs are seen when either wheat germ or reticulocyte cell-free systems are used. As expected, however, more high molecular weight polypeptides are detected when translations are performed in the reticulocyte system because of the lower proportion of incomplete polypeptide formation (Pelham & Jackson, 1976).

Template activity of different preparations of nonpolysomal RNA varied between a minimum of 53% and a maximum of 74% of the total in experiments. However, the template activity of a particular preparation of RNA was found to be independent of the cell-free system used.

Much evidence has been presented which favors the hypothesis that most transcription in the testis occurs before spermiogenesis and haploid cells transcribe relatively little RNA [Loir, 1971; Moore, 1971; Kierszenbaum & Tres, 1974, 1975; Söderstrom & Parvinen, 1976; for review see Monesi et al. (1978)]. Since postmeiotic cells have been shown to be active in protein synthesis (Monesi, 1964; Turkington & Mujumder, 1975; Boitani et al., 1980), this protein synthetic activity may represent the expression of preserved or long-lived mRNA present in haploid cells (Monesi, 1965; Söderstrom, 1976; Geremia et al., 1977, 1978) or newly synthesized mRNA. One example of stored testis mRNA, protamine messenger, synthesized during meiotic prophase, has been elegantly characterized by Dixon and co-workers [for review see Iatrou & Dixon (1978)]. They conclude that in the trout the messenger RNAs which encode protamine are transcribed in the primary spermatocyte and stored in RNP particles until translational "activation" in round spermatids. More recently, evidence for the synthesis of poly(A)+ RNA in postmeiotic cells has been demonstrated (D'Agostino et al., 1978; Erickson et al., 1980a), and postmeiotic increases for the amounts of mRNA encoding two proteins, protamine-like histone and phosphoglycerate kinase-2, that are expressed during spermiogenesis have been reported (Erickson et al., 1980b). Such evidence suggests that one might expect to find both transcriptional and translational methods of regulation during spermatogenesis.

The mechanism by which this nonpolysomal mRNA becomes polysome bound (if it does) is not known. It has previously been postulated that addition of poly(A) (Slater et al., 1973; Wilt, 1973) or of a 5′,7-methylguanine cap (Muthukrishnan et al., 1975) or some other structural alteration (Lodish, 1974) of nonpolysomal mRNA might mediate its loading onto the polysomes. Poly(A)− sea urchin histone mRNA has since been shown to lack poly(A) both in the egg and after loading onto polysomes in the embryo (Nemer et al., 1975; Lifton et al., 1976), and both poly(A)+ and poly(A)− protamine mRNAs are found on the polysomes in trout testis Iatrou & Dixon, 1977). Other studies with nonpolysomal maternal mRNA have similarly suggested that it is capped, methylated, and translatable (Hickey et al., 1976a,b). On the other hand, Lodish (1974) has advanced the hypothesis that the RNA from the nonpolysomal fraction itself may be less capable of effecting ribosome binding in vitro as compared with polysomal RNA. This hypothesis has been supported by mixing experiments between polysomal and nonpolysomal RNAs from sea urchin embryos (Rudensey & Infante, 1979). Our results from testis indicate that different translational efficiencies cannot readily explain why some mRNAs are polysome bound and others are not, since we find both populations of mRNAs are translated when deproteinized RNA from unfractionated testis cytoplasm is analyzed. Perhaps competitive mixing experiments between testis polysomal and nonpolysomal RNA analogous to those performed with sea urchin RNA by Rudensey & Infante (1979) could resolve this question. However, this would not answer whether the genesis of differential translational efficiency is the structure of the *mRNA* or of the *mRNP*. A test to see whether nonpolysomal mRNPs may not compete as effectively as polysomal *mRNP*s for ribosome binding sites is needed to clarify this point.

Recent experiments with *Spisula* suggest that a protein or other phenol-soluble moiety associated with the nonpolysomal RNA might be a negative regulator of ribosome binding (Vincent et al., 1977; Rosenthal et al., 1980). Although we do not observe many differences in the relative amounts of polypeptides encoded by extracted and unextracted cytoplasm from testis, one cannot dismiss the presence of such a regulatory mechanism in the testis. It is possible that the isolation procedures we employ for cell-free translation release specific regulatory factors that are normally bound to certain mRNAs.

Lastly, one must consider the possibility that the cellular cytoskeleton plays a role in the regulation of gene expression during spermatogenesis (Burr et al., 1980). The temporal sequestration of nonpolysomal RNA by selective attachment to the cellular cytoskeleton of testicular cells merits investigation.

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