Ramachandran, J. (1971), *Anal. Biochem. 43,* 227–233.
Rodbell, M., Birnbaumer, L., and Pohl, S. (1970), *J. Biol. Chem. 245,* 718–722.
Rodbell, M., Birnbaumer, L., Pohl, S. L., and Karns, H. M. J. (1971), *J. Biol. Chem. 246,* 1877–1882.
Rodbell, M., Lin, M. C., and Salomon, Y. (1974), *J. Biol. Chem. 249,* 59–65.
Sussman, K. E., and Vaughan, G. E. (1967), *Diabetes 16,* 449–454.
Thompson, W. J., and Appleman, M. M. (1971), *Biochemistry 10,* 311–316.
Thompson, W. J., Williams, R. H., Little, S. A. (1973), *Biochim. Biophys. Acta 302,* 329–335.
Turtle, J. R., and Kipnis, D. M. (1967), *Biochem. Biophys. Res. Commun. 28,* 797–800.
Umbreit, W. W., Buvis, R. H., and Stauffer, J. F. (1945), Manometric Techniques, Minneapolis, Minn., Burgess Publishing Co., p 149.
Wizeman, V., Schulz, I., and Simon, B. (1973), *Biochim. Biophys. Acta 307,* 366–371.
Wolff, J., and Cook, G. H. (1973), *J. Biol. Chem. 248,* 350–356.

Determination of Molar Ratios of Vesicular Stomatitis Virus Induced RNA Species in BHK_{21} Cells†

Luis P. Villarreal, Michael Breindl,‡ and John J. Holland*

ABSTRACT: A modified procedure for analysis of RNA in denaturing formamide–polyacrylamide slab gels containing 6 M urea is described. Using this technique, in conjunction with fluorographic analysis, we determined molecular weights and molar ratios of the various vesicular stomatitis virus (VSV) induced RNAs in BHK_{21} cells. A comparison of the molar ratios of virus-specific mRNAs and their putative protein products in these cells suggests that there is little, if any, translational control of viral gene expression during acute VSV infection.

A technique for the electrophoresis of RNA in formamide–acrylamide gels has previously been described in detail (Pinder et al., 1974). This technique worked well for tube gel analysis of RNA, but attempts to apply it to slab gel electrophoresis followed by fluorographic analysis of tritiated RNA (Bonner and Laskey, 1974) met substantial difficulties in this laboratory. The modifications described here facilitate the handling of low percentage acrylamide gels containing formamide and allow quantitative fluorographic analysis (Laskey and Mills, 1975) of tritiated RNAs of high molecular weight. The method is applied to an analysis of virus-induced RNA species in vesicular stomatitis virus (VSV[1]) infected cells. VSV is an enveloped negative strand RNA virus with an associated transcriptase. RNA synthesized in VSV-infected cells in the presence of actinomycin D is predominantly virus-specific "plus" (messenger) strand (for review, see Wagner, 1975). Recently, electrophoretic separation of VSV-specific RNAs was accomplished under denaturing conditions (Rose and Knipe, 1975; Grubmann et al., 1975). In vitro translation of these RNAs allowed identification of the coding capacity of four of the five VSV-specific mRNAs (Knipe et al., 1975). The remaining RNA species (28S) is thought to code for the large (L) VSV structural protein (Morrison et al., 1974). In infected cells all five VSV-specific proteins are synthesized throughout the infectious cycle, in about the same relative proportion (Mudd and Summers, 1970), but the molar amounts of the individual proteins differ greatly from one another, suggesting a rather stringent regulation of VSV protein synthesis. The data presented in this paper suggest that this regulation occurs at the level of transcription rather than translation.

Materials and Methods

Materials. 99% formamide was purchased from Mallinckrodt. Acrylamide was from BDH. *N,N'*-Methylenebisacrylamide and *N,N,N',N'*-tetramethylethylenediamine (Temed) were from Eastman. Other chemicals were reagent grade.

Deionization of Formamide–Urea. Urea was dissolved in formamide to a final concentration of 6 M. The solution was then mixed with mixed-bed, ion-exchange resin (Amberlite coarse MB-1, 8 g/100 ml) and stirred for 30 min at room temperature during which the conductivity of the solution dropped to below 5 μmho. The formamide–urea was filtered and stored in the dark at 5 °C. The solution could be stored for up to 10 days before conductivity rose to undesirable levels.

Preparation of RNA Gels. Gels were 2.8% (w/v) polyacrylamide with 2.38% acrylamide and 0.4% *N,N'*-methylenebisacrylamide. In 30 ml of the deionized formamide–urea solution was dissolved 0.715 g of acrylamide in 0.126 g of *N,N'*-methylenebisacrylamide. To this solution was then added 0.11 g of barbital and 0.072 ml of Temed. The apparent pH was adjusted to 9.0 using a standard glass elec-

† From the Department of Biology, University of California, San Diego, La Jolla, California 92093. *Received October 21, 1975.* This work was supported by Grant No. 10802 from the National Cancer Institute and by a research fellowship from Deutsche Forschungsgemeinschaft.

‡ Present address: Heinrich-Pette-Institut, 2 Hamburg 20, Germany.

[1] Abbreviations used: VSV, vesicular stomatitis virus; Temed, *N,N,N',N'*-tetramethylethylenediamine; L VSV structural protein, large VSV structural protein; G protein, glycosylated protein; N protein, nucleocapsid protein; NS protein, minor protein; M protein, matrix protein.

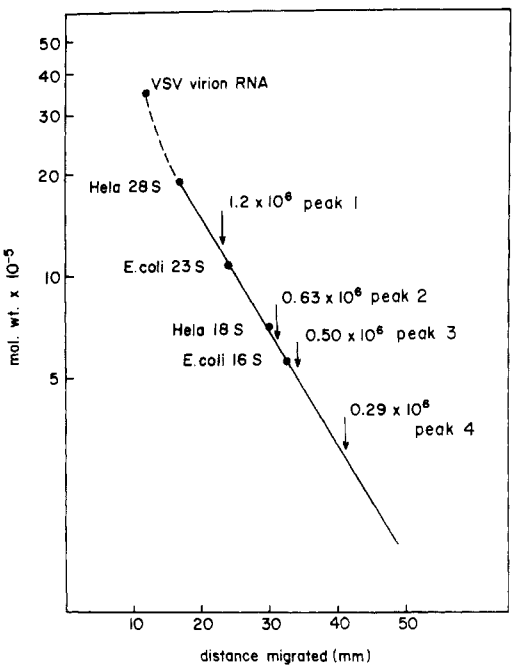

FIGURE 1: Molecular weight calibration for RNA with distance migrated in formamide–urea polyacrylamide gels. RNA gels were prepared and run as described in Materials and Methods. Positions of marker RNAs are indicated by points (●). Molecular weights were 1.9×10^6, 1.07×10^6, 0.71×10^6, and 0.55×10^6 for HeLa 28S, *E. coli* 23S, HeLa 18S, and *E. coli* 16S, respectively (McConkey and Hopkins, 1969; Stanley and Bock, 1965). Positions of VSV-induced RNA species are indicated by arrows. Calibrations were done using different tracks of the same slab gel. RNAs were labeled with [^{3}H]uridine and their positions in the gel determined by fluorography as described in Materials and Methods. The value used for VSV virion RNA molecular weight was 3.6×10^6. Estimates of this value range from 3.4 to 3.8 $\times 10^6$. For references, see Wagner (1975).

trode and a calomel reference electrode containing saturated aqueous potassium chloride by dropwise addition of 1 N HCl to the rapidly stirring formamide solution. This solution (prior to the addition of ammonium persulfate) was used to wet a sheet of Whatman no. 1 paper that had previously been cut to fit the unnotched glass, slab-gel plate (13 × 15 cm in our procedure). The paper was aligned on the glass plate and wet with about 4 ml of the formamide–acrylamide solution. Bubbles were removed by rolling a pipette over the paper. Spacers (2.0 mm) and the notched, slab-gel glass plate were aligned on the wet plate and then sealed with 1.5% agarose containing 1% sodium dodecyl sulfate. Freshly prepared 18% w/v ammonium persulfate (0.1 ml) was then added to the remaining acrylamide–formamide solution. The gel was poured and the combs (1.9-mm thick with 6-mm well width) set in place. Polymerization was complete in 30 min. The wells were washed and filled with 0.02 M barbital in formamide (pH 9.0). RNA samples (about 1 mg/ml) were dissolved in 6 M urea–99% formamide and denatured at 70 °C for 1 min. Trace amounts of bromophenol blue tracking dye were added and samples layered in the wells (15–30 μl, containing about 20 000 cpm). Gels were run at 7 mA with recirculation of the reservoir solution (0.02 M NaCl in H_2O) for 16–20 h at which time the tracking dye had reached the end of the gel, just in front of 4S RNA. The gels were removed from the plates and the RNA was fixed by immersing the gels in 10% formaldehyde solution at 23 °C for 20 min followed by a water rinse.

Tritium Fluorography. Processing of the gels for tritium fluorography was done according to the methods of Bonner and Laskey (1974) with the following modifications for RNA gels. Ten percent rather than 16% 2,5-diphenyloxazole in Me_2SO was used for 2-mm thick gels. Precipitation of 2,5-diphenyloxazole in the gel in preparation for drying was done with 40% v/v ethanol in water to eliminate gel distortion. Gels were dried under a heat lamp in vacuo covered with a low porosity plastic wrap and supported by Whatman 3MM paper on a porous polyethylene pad. Fluorographs were done on Kodak RP Royal "X-Omat" film with 24 h of exposure at −70 °C using preexposed film according to the methods of Laskey and Mills (1975) for quantitative film response.

Preparation of VSV-Induced RNA. Monolayers of BHK_{21} hamster kidney cells were used for virus growth. Cells were grown in Eagle's minimum essential medium plus 7% (v/v) calf serum. VSV Indiana strain was originally obtained from Dr. John Mudd (Mudd and Summers, 1970) and cloned six consecutive times. Virus was absorbed on cell monolayers for 30 min at 37 °C (MOI ≃ 100). Actinomycin D (5 μg/ml, Calbiochem) was added at 2.5 h postinfection. [^{3}H]Uridine was added to a final concentration of 25 μCi/ml in enough media to cover the monolayer at 3.0 h postinfection. At 5 h post-infection, the supernatant was poured off the cells and the cells were extracted with hot (60 °C) 1:1 phenol–chloroform and an equal volume of aqueous 50 mM Tris-HCl (pH 9.0) and 0.15 M NaCl. The RNA was ethanol precipitated (two times), and the dried pellets were dissolved in a small volume of 6 M urea in formamide.

In Vivo Labeling of VSV Proteins. At 4 h postinfection with VSV the cells were washed with medium free of serum and amino acids and pulse labeled for 30 min with 30 μCi/ml of ^{14}C-amino-acid-labeled mix from NEN (No. WEC-445), specific activity approximately 0.012 mCi/mmol with an amino acid composition and concentration approximately that of Eagle's medium. The cells were then washed with isotonic saline and lysed in 10 mM Tris-HCl buffer, pH 7.6, containing 2.5 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 0.5% NP 40, and 0.5% sodium deoxycholate. The cell lysate was centrifuged for 15 min at 2000*g* and proteins precipitated from the supernatant with 9 volumes of acetone at −20 °C overnight. The sediment was air dried and dissolved in electrophoresis buffer.

Polyacrylamide Gel Electrophoresis of Proteins. Protein electrophoresis was performed according to the method of Laemmli (1970) as described before (Breindl and Holland, 1975).

Results

Modifications of the Gel Electrophoresis Procedure. Using the original formula of Pinder et al. (1974), we were unable to prepare formamide–acrylamide slab gels for electrophoresis and fluorographic analysis of tritiated RNAs for two reasons. (a) In our hands low percentage (2.5–3% w/v) polyacrylamide gels, which are required for analysis of high molecular weight RNAs, did not reproducibly polymerize into firm gels in the presence of 99% formamide. For reasons we do not understand, this difficulty was eliminated by using 6 M urea–formamide rather than formamide as a solvent. (b) The poor mechanical strength of 2.5–3% acrylamide gels led to difficulties in separation of the glass plates after electrophoresis and in subsequent handling of the gel slabs during preparation for fluorography. This problem

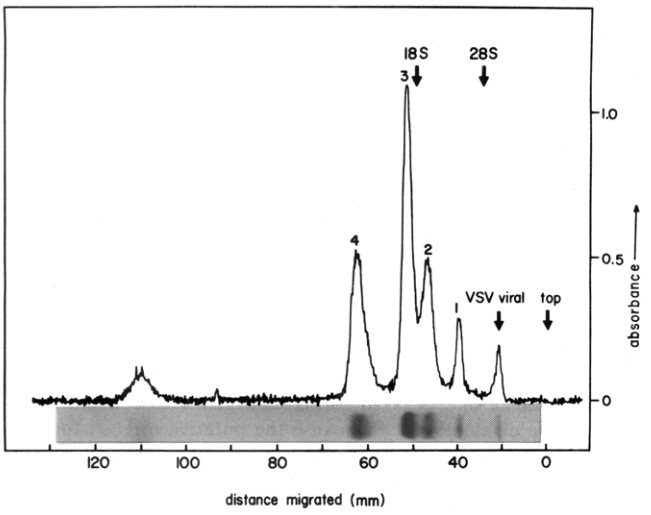

FIGURE 2: Densitometer tracing of a denaturing formamide–urea gel electrophoretic image on a x-ray film fluorograph of VSV-induced [^{3}H]uridine labeled RNA species in infected cells. A Joyce-Loebel Micro densitometer was used to trace the grain absorbance on an x-ray film of a gel pattern prepared as described in Materials and Methods. Fluorographic image was obtained by exposing the gel (40 000 cpm per well) overnight at −70 °C employing pre-exposed film as described by Laskey and Mills (1975) to assure linear response. Several exposure times were tried and our optimal light exposure was employed to prevent overexposure in any peak area.

was overcome by polymerizing the gels on Whatman no. 1 filter paper, as described in Materials and Methods. The presence of the paper resulted in the desired increased mechanical stability of the gel and seemed not to affect the mobility of the analyzed RNAs or the resolving power of the gel. Elution of small RNA species from the gel during processing for fluorography was prevented by fixation in a 10% (v/v) formaldehyde solution prior to the first Me_2SO treatment.

Determination of Molecular Weights and Molar Ratios of Virus-Specific RNAs in VSV-Infected BHK$_{21}$ Cells. Figure 1 shows the separation of various RNAs of known molecular weights and the relative positions of the various virus-specific RNAs from VSV-infected cells. There was a consistent linear relationship between the electrophoretic mobility and the logarithm of the molecular weight for RNAs with molecular weights of up to 1.9×10^6 daltons, when 2.8% gels were used. VSV-specific RNA, under totally denaturing conditions, was comprised of five distinct species, the largest of which co-migrated with virion RNA (ca. 3.6×10^6 daltons). The apparent molecular weights of the remaining four species (designated as peaks 1, 2, 3, and 4), were 1.2×10^6, 0.63×10^6, 0.50×10^6, and 0.29×10^6 daltons, respectively. These estimates are in good agreement with those reported by Rose and Knipe (1975) except that their estimate of peak 1 RNA was 1.65×10^6 daltons, using 3.75% gels.

Fluorograms and densitometer tracings of [^{3}H]uridine-labeled VSV-specific RNAs separated by gel electrophoresis are shown in Figure 2. It is emphasized that we used the procedure of Laskey and Mills (1975) which allows a quantitative determination of radioactivity in polyacrylamide gels. The RNA profile shown in Figure 2 was very reproducible and did not change substantially during the infectious cycle or as a function of the multiplicity of infection (not shown). Peak 2, 3, and 4 RNAs have been identified as mRNAs by in vitro translation (Knipe et al., 1975), where

Table I: Molar Ratios for VSV Induced RNAs.[a]

RNA Species	Mol Wt[b]	Putative Gene Product	Molar Ratio[c]
Virion	3.6×10^6		1.1
Peak 1	1.2×10^6	L protein	5.6
Peak 2	0.63×10^6	G protein	36
Peak 3	0.50×10^6	N protein	100
Peak 4	0.29×10^6	NS and M proteins	107

[a] Peak areas from Figure 2 and corrected molecular weights from Figure 1 were used to calculate molar ratios. Peak areas were determined by plotting Figure 2 with an expanded x axis on uniform weight bond paper, and each peak was cut out and weighed. [b] Molecular weights were corrected for poly(A) content of 100 nucleotides (Rose and Knipe, 1975). [c] Molar ratios were normalized to 100 equiv of peak 3.

peak 2 RNA coded for the glycosylated (G) protein, peak 3 RNA for the nucleocapsid (N) protein, and peak 4 RNA coded for two proteins, the minor (NS) protein and the matrix (M) protein. A similar result was obtained by Both et al. (1975) who separated the VSV-specific mRNAs by sucrose gradient centrifugation.

Table I gives the molar ratios for the various RNAs using molecular weights from Figure 1 and peak areas from Figure 2. The values are corrected by subtraction of 100 nucleotides of poly(A) per RNA molecule and normalized to 100 equiv of peak 3 RNA. Assuming the assignment of coding functions to be correct, the relative amounts of the mRNAs are 5.6 L, 36 G, 100 N, and 107 NS plus M. This is in contrast to the conclusion by Stamminger and Lazzarini (1974) that the mRNAs for the individual VSV proteins may be present intracellularly in equimolar concentrations.

Molar Ratios of Virus-Specific Proteins in VSV-Infected BHK$_{21}$ Cells. The cytoplasmic extract prepared as described in Materials and Methods was found to contain 97.3% of the total Cl_3CCOOH precipitable ^{14}C-amino-acid-labeled counts per minute; 1% of the label was found in the medium and 1.7% in the nuclear pellet. Gel analysis of labeled proteins in the nuclear pellet showed them to be similar in composition to those found in the cytoplasm except for an increased ratio of L protein (not shown).

An analysis by gel electrophoresis of ^{14}C-amino-acid-labeled, virus-specific proteins from VSV-infected cell cytoplasm and a densitometer tracing of the fluorographic image are shown in Figure 3. We calculated the molecular weights of the individual VSV-specific proteins by co-electrophoresis with proteins of known molecular weight and found them to be 198 000 (L protein), 6000 (G protein), 5000 (NS protein), 45 000 (N protein), and 30 000 daltons (M protein) (data not shown). Similar values were obtained by Wagner et al. (1972) and Both et al. (1975). Using these molecular weights and the peak areas from Figure 3, the molar ratios for VSV-specific proteins were calculated.

When normalized to 100 equiv of N protein, the relative amounts of the individual proteins were 6.9 L, 34 G, 100 N, 77 M, and 53 NS (Table II). These values show a striking accordance with the molar ratios calculated for the corresponding mRNAs (Table II) and suggest strongly that there is little or no translational regulation, although there is obviously some form of transcriptional regulation. We have observed no significant deviation of protein molar ratios at early or later times of infection in agreement with

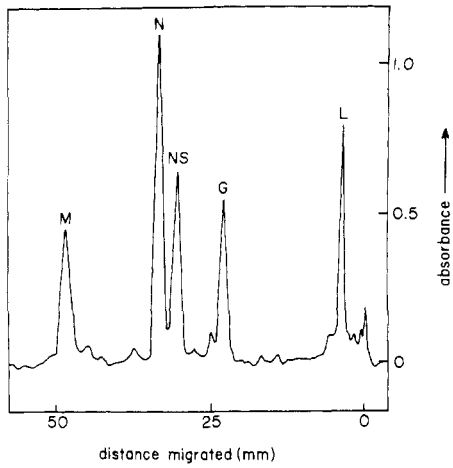

FIGURE 3: Densitometer tracing of an autoradiographic x-ray film image of a sodium dodecyl sulfate-gel electropherogram of VSV-induced, ^{14}C-labeled proteins in infected cells. Proteins were prepared and gels run as described in Materials and Methods (80 000 cpm were used per run). The autoradiographic image was obtained after 6 h of exposure at −70 °C employing preexposed film as described by Laskey and Mills (1975).

Mudd and Summers (1970) so these values are representative of the entire viral growth cycle.

Discussion

Our modified technique for RNA electrophoresis in polyacrylamide slab gels using formamide-urea as a solvent, in conjunction with fluorographic methods for the detection of tritium-labeled compounds by autoradiography (Bonner and Laskey, 1974; Laskey and Mills, 1975), allows a sensitive quantitative analysis of tritiated RNAs of high molecular weight under totally denaturing conditions, and co-electrophoresis of several known and unknown RNA species labeled with the same or different radionuclides. Using this technique, our molecular weight determinations of virus-specific RNAs extracted from VSV-infected BHK_{21} cells are, within experimental errors, in agreement with those reported by others and with the expected capacity of the different mRNAs (Rose and Knipe, 1975; Knipe et al., 1975; Both et al., 1975; Grubmann et al., 1975) with the exception of peak 1 RNA (Figure 1, Table II). The value obtained for peak 1 RNA, under conditions of a clear linear relationship between electrophoretic mobilities and logarithm of the molecular weights of RNA species with molecular weights of up to at least 1.9×10^6 daltons (Figure 1) was very reproducible at 1.2×10^6 daltons. An RNA species with identical molecular weight is synthesized, under appropriate conditions, by the VS virion associated transcriptase in vitro (Breindl and Holland, in preparation). We therefore think that the 1.2×10^6 molecular weight determination is accurate and we cannot explain the difference between this value and that of Rose and Knipe (1975). Even more intriguing is the function of peak 1 RNA. In vitro translation studies have so far failed to prove any messenger function of in vitro synthesized peak 1 RNA (Breindl and Holland, in preparation), nor is there convincing evidence that this RNA, isolated from infected cells, serves as mRNA. Its putative identification as L protein mRNA (Table II) is in striking disagreement with its coding capacity (a protein with a molecular weight of maximal 130 000 daltons). A similar situation exists for the NS protein mRNA (peak 4 RNA), which has a molecular weight of 0.29×10^6 daltons, corresponding to a coding capacity of

Table II: Molar Ratios for VSV Induced Proteins.[a]

Protein	Mol Wt	Protein Molar Ratio	RNA Molar Ratio
L	198 000	6.9	5.6
G	66 000	34	36
N	47 000	100	100
M	30 000	53	107[b]
NS	52 000	77	*b*
NS_{cor}[c]	32 000[d]	48[e]	*b*

[a] Peak areas from Figure 3 were used to calculate molar ratios as described in Figure 2. Molecular weights of VSV proteins in our gels were the same as those of Both et al. (1975). [b] NS and M mRNAs run as one peak in formamide gels (Rose and Knipe, 1975; Knipe et al., 1975). [c] Because the putative mRNA for NS protein does not agree in apparent size with the apparent size of NS protein (see text), we have included a corrected protein size and corrected molar ratio for comparison with the uncorrected. [d] Corrected for mRNA size. [e] Corrected.

a 32 000 dalton protein, whereas the apparent molecular weight of the NS protein has been estimated to be between 40 000 and 52 000 daltons, depending on the buffer system used for gel electrophoresis (Obijeski et al., 1974). Similar differences in molecular weight determinations have been reported for the L protein (Wagner et al., 1972; Obijeski et al., 1974), and it may be that, for unknown reasons, molecular weight determinations of the VSV L and NS proteins have been inaccurate and that peak 1 RNA is indeed the L protein mRNA. Alternatively, it is also possible that the L and LS protein mRNA contains some denaturation-resistant secondary structure and migrates anomalously fast in our gel system, which would lead to an underestimate of its molecular weight and an overestimate of its relative molar abundance.

Some evidence does in fact exist to support this latter explanation. Knipe et al. (1975) showed that the nucleotide sequence complexity of band 4 RNA (NS and M protein mRNAs) has the capacity to code for protein(s) of $0.72–0.98 \times 10^6$ daltons. The combined molecular weight of NS and M proteins (as obtained from our protein gels) is 0.82×10^6. An unequivocal answer to this question has to await the translation in vitro of peak 1 RNA into its protein product and sequence determination of the NS and L protein mRNAs.

The molar ratios of the individual VSV proteins appear to be, within experimental error, almost identical with the molar ratios of their mRNAs (Table II). The close agreement of the molar ratios of the various VSV mRNAs with their protein products, as well as the obvious differences in the rate of synthesis of the individual VSV mRNAs in vivo (Figure 2, Table I) and in vitro (Roy and Bishop, 1972; Breindl and Holland, in preparation), justifies the conclusion that the regulation of the viral gene expression during acute VSV infection occurs mainly, if not exclusively, on the level of transcription. The mechanism of this transcriptional regulation is at present unknown.

Note Added in Proof

L. A. Ball and C. N. White ((1976), *Proc. Natl. Acad. Sci. U.S.A. 73*, 442) have proposed on the basis of uv target sizes that the gene order along the VSV template for transcription is 3′-N-NS-M-G-L-5′, and that these five genes are probably transcribed from a single initiation point.

Since the data presented above show decreasing protein and mRNA abundances in the same order, the "transcriptional regulation" observed here may merely reflect a probability of "fall-off" of each transcriptase molecule as it proceeds along its template beyond each gene.

References

Bonner, W. M., and Laskey, R. A. (1974), *Eur. J. Biochem. 46,* 83.
Both, G. W., Moyer, S. A., and Banerjee, A. K. (1975), *J. Virol. 15,* 1012.
Breindl, M., and Holland, J. J. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2545.
Grubmann, N. J., Moyer, S. A., Banerjee, A. K., and Ehrenfeld, E. (1975), *Biochem. Biophys. Res. Commun. 62,* 531.
Kang, C. Y., and Prevec, L. (1971), *Virology 46,* 678.
Knipe, D., Rose, J. K., and Lodish, H. F. (1975), *J. Virol. 15,* 1004.
Laemmli, U. K. (1970), *Nature (London) 227,* 680.
Laskey, R. A., and Mills, A. D. (1975), *Eur. J. Biochem. 56,* 335.
McConkey, E. H., and Hopkins, J. W. (1969), *J. Mol. Biol. 39,* 545.
Morrison, T., Stampfer, M., Baltimore, D., and Lodish, H. F. (1974), *J. Virol. 13,* 62.
Mudd, J. A., and Summers, D. F. (1970), *Virology 42,* 328.
Obijeski, J. F., Marchenko, A. T., Bishop, D. H. L., Cann, B. W., and Murphy, F. A. (1974), *J. Gen. Virol. 22,* 21.
Pinder, J. C., Staynov, D. Z., and Gratzer, W. B. (1974), *Biochemistry 13,* 5373.
Rose, J. K., and Knipe, D. (1975), *J. Virol. 15,* 994.
Roy, P., and Bishop, D. H. L. (1972), *J. Virol. 9,* 946.
Stamminger, G., and Lazzarini, R. A. (1974), *Cell 3,* 85.
Stanley, W. M., Jr., and Bock, R. M. (1965), *Biochemistry 4,* 1302.
Wagner, R. R. (1975), in *Compr. Virol. 4,* 1.
Wagner, R. R., Kiley, M. P., Snyder, R. M., and Schnaitmann, C. A., (1972), *J. Virol. 9,* 672.

Interactions of Mercury and Copper with Constitutive Heterochromatin and Euchromatin in Vivo and in Vitro†

Sara E. Bryan,* Sam J. Simons, Douglas L. Vizard,‡ and Kenneth J. Hardy‡

ABSTRACT: Mouse liver nuclei were fractionated into (condensed) heterochromatin and (noncondensed) euchromatin by differential centrifugation of sonicated nuclei. The fractions were subsequently characterized as unique nuclear species by thermal denaturation derivative profile analysis, which revealed the heterochromatin fraction enriched in satellite DNA and by endogenous metal content, which displayed partitioning of mercury in euchromatin over heterochromatin by a 10:1 ratio, with a comparatively uniform distribution of copper in both fractions. Fractionation of nuclei following in vivo challenge with copper showed enrichment of copper in heterochromatin, relative to euchromatin, while in vivo exposure to mercury resulted in a 20-fold accumulation of mercury in euchromatin, relative to heterochromatin. Using gel filtration and equilibrium dialysis to measure in vitro binding under relatively physiologic conditions of pH (6.0–7.0) and ionic strength (standard saline citrate or saline), the condensed and noncondensed chromatin fractions exhibited binding specificities toward mercury and copper similar to that observed in the in vivo metal challenge experiments. The level of mercury which binds to euchromatin in vitro, when measured either in physiologic [standard saline citrate (SSC)] or in dilute (1:100 SSC) salt solutions, was comparable (approximately 3 μg of Hg/mg of DNA) to that of in vivo euchromatin-bound mercury after 1 month of challenge with dietary metal. In contrast, copper showed little or no preference for the nuclear fractions in dilute salt solutions and displayed patterns which mimic in vivo binding only at higher ionic strengths (saline). Removal of proteins from the chromatin fractions resulted in a loss of binding specificity toward both metals. Therefore, the binding selectivity of condensed and noncondensed chromatin toward both mercury and copper appears to arise from protein or from protein–DNA associations. The state of chromatin condensation is especially critical in the case of copper.

A growing body of evidence supports the view that controlling elements which regulate the activity of genes in eukaryotic cells reside in chromatin, the complex interphase chromosomal material (Stein et al., 1974; Stein and Farber, 1972; Gilmour, 1974; Wilhelm et al., 1971). The evidence implicates chromosomal proteins and, in particular, nonhistone proteins as the regulatory elements (Stein et al., 1974; Gilmour, 1974; and Spelsberg et al., 1972). However, the fact that chromatin contains metals (along with DNA, RNA, and histone and nonhistone protein) which display unique binding affinities for each of the nuclear constituents could be important in understanding the structure of the genome and how chromosomal proteins regulate its function.

† From the Department of Biological Sciences, University of New Orleans (S.E.B. and S.J.S.), New Orleans, Louisiana, and the Department of Biochemistry and Biophysics (D.L.V. and K.J.H.), M.D. Anderson Hospital and Tumor Institute, University of Texas, Houston, Texas. *Received June 18, 1975.* This investigation was supported by a NIH research Grant 1 R01 ES00802-0 from National Institute of Environmental Health Sciences.

‡ Present address: Department of Biochemistry and Biophysics, M.D. Anderson Hospital and Tumor Institute, University of Texas, Houston, Texas.