Sequence Complexity of Polyadenylated RNA Obtained from Rat Brain Regions and Cultured Rat Cells of Neural Origin†

B. B. Kaplan,‡ B. S. Schachter,§ H. H. Osterburg, J. S. de Vellis,¶ and C. E. Finch*

ABSTRACT: The nucleotide base sequence complexity of total poly(A) RNA from rat tissues and cultured cells of neural origin was measured by RNA-driven hybridization reactions with nonrepetitive [^{3}H]DNA. At saturation, liver and whole brain poly(A) RNAs were complementary to an average of 6.8 and 12.3%, respectively, of the nonrepetitive DNA. Assuming asymmetric transcription, the complexity of liver and brain poly(A) RNAs was equivalent to 3.6 and 6.5×10^8 nucleotides, respectively. The complexity of whole brain poly(A) RNA was similar to that from major functionally distinct brain regions: cerebellum, hypothalamus, hippocampus, and cerebral cortex. Considerations of experimental variance suggest that total poly(A) RNA sequence overlap between major brain regions is substantial and may be ≥80%. Cultured cells of neural origin had poly(A) RNA complexities which were intermediate between liver and brain in the order: liver < primary glial cultures from 3-day-old brain (predominantly astrocytes) < neuroblastoma (B104) < glioma (C6) < brain. Hybridization of combinations of RNA from the sources indicates extensive similarities in poly(A) RNA sequences between neuroblastoma, glioma, and glial cells and suggests that most liver and neuroblastoma poly(A) RNA sequences are a subset of those in the brain. Comparison of data from this and other studies suggests that cultured cells of neural origin may, like the fully differentiated brain, have an unusually complex RNA population. If these findings reflect the situation in vivo, the large complexity and homology of neuronal and glial RNA could underlie the similarities in poly(A) RNA sequence complexity observed between different brain regions. The hybridization kinetics of poly(A) RNA from whole brain and from functionally distinct brain regions were comparable and revealed at least two kinetic classes. The most slowly hybridizing RNA component hybridized with a pseudo-first-order rate constant of 1.9×10^{-4} L mol^{-1} s^{-1} and contained 70% of the poly(A) RNA complexity. The fraction of the poly(A) RNA driving the slow reaction was 4% of its mass. From these figures it is calculated that each slowly hybridizing poly(A) RNA sequence is present in about 0.03 copy per brain cell. This suggests that each brain region studied contains at least 30 different cell classes distinguished by poly(A) RNA sequence content.

The nucleotide base sequence complexity of brain RNA has been estimated by hybridization experiments in which nonrepetitive DNA is reacted with excess total RNA (Brown and Church, 1971; Grouse et al., 1972) or nuclear RNA (Hahn and Laird, 1971; Cutler, 1975). Results of these studies suggest that the transcriptional diversity in brain was significantly greater than in other tissues or organs. Recent measurement of the sequence complexity of brain nuclear RNA indicates that 30–40% of the nonrepetitive genome is transcribed in this organ, assuming asymmetric transcription (Bantle and Hahn, 1976; Chikaraishi et al., 1978; Grouse et al., 1978). In contrast, estimates of the nonrepetitive sequence representation in nuclear RNA from other tissues are substantially lower, e.g., 22% in liver and 9–10% for kidney, spleen, and thymus (Chikaraishi et al., 1978).

Correspondingly, brain poly(A) mRNA hybridizes to two to three times more nonrepetitive DNA than does mRNA of other tissues or organs. Assuming asymmetric transcription, rodent brain poly(A) mRNA has a sequence complexity of approximately 1.4×10^8 nucleotides, a value equivalent to the transcription of 50 000–100 000 average-size structural genes (Bantle and Hahn, 1976; Grouse et al., 1978). The hybridization kinetics of DNA complementary (cDNA) to mouse brain cytoplasmic poly(A) RNA (Ryffel and McCarthy, 1975) and polysomal poly(A) RNA (Young et al., 1976) also indicate that more genes are expressed in brain than in other organs.

The striking complexity of brain RNA could result from the summation of RNA complexities from the numerous cell types present in the adult organ. Alternatively, cells of neural origin may have similar but unusually complex RNA populations. One approach to this question involves comparison of RNA from brain regions which differ greatly in structure and function. In an early hybridization study (Hahn, 1973), no quantitative or qualitative differences were detected in nuclear RNA from monkey cerebral cortex or cerebellum. In contrast, Grouse et al. (1973), using limited amounts of material from human brain, suggest regional differences in the hybridization of total RNA to nonrepetitive DNA. Another approach to elucidating the origins of the high complexity of brain RNA is to examine various cell types of neural origin. In this regard, total RNA from a mouse neuroblastoma and two human astrocytomas hybridized to two to three times less nonrepetitive DNA than did total brain RNA (Grouse et al., 1973).

The present study compares the complexity of total poly(A) RNA from several brain regions, under conditions which permit a closer approach to maximum hybridization values than achieved in early studies. The major brain regions studied (cerebral cortex, hippocampus, hypothalamus, and cerebellum) were selected because of their widely differing cell compositions, functional characteristics, and cytoarchetectonic

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‡ Present address: Department of Anatomy, Cornell University Medical College, New York, N.Y. 10021.

§ Present address: Metabolic Research Unit, University of California Medical School, San Francisco, Calif. 94143.

¶ Present address: Department of Anatomy, University of California at Los Angeles, Los Angeles, Calif. 90024.

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organization (Pearson and Pearson, 1976). The extent to which different cell types contribute to the overall transcriptional diversity of brain was investigated using primary glial cell cultures (predominantly astrocytes) and cloned cell lines derived from chemically induced CNS tumors, the C6 glioma and B104 neuroblastoma. Liver was also examined to provide a comparison with a tissue of known lower RNA complexity.

In an effort to minimize RNA degradation that can occur during the prolonged periods required for brain dissection and isolation of specific cell organelles, total poly(A) RNA was obtained directly from frozen tissue. As demonstrated by Bantle and Hahn (1976), poly(A) RNA isolated from brain total RNA or nuclear RNA has the same complexity. Thus, the total poly(A) RNA complexity estimates reported in this work can be taken to indicate the complexity of nuclear poly(A) RNA transcription.

Materials and Methods

Animals and Brain Dissection. Male Sprague-Dawley or Fischer 344 rats (60–70 days old) were maintained in group cages under 12-h light–dark cycles at 24 °C, with water and Purina Lab Chow available ad libitum. Rats were sacrificed by decapitation, and brains (2 g) were rapidly removed without the pituitary and olfactory bulbs and dissected on a chilled surface. The cerebellum (350 mg) was excised at its peduncles. The cerebral cortex (200 mg) including the corpus callosum was spread back from the underlying structures and excised laterally at the rhinal sulcus. The hippocampus (70 mg) was then removed. Cuts into the ventral brain, circumscribing the external margins of the hypothalamus to 2-mm anterior to the optic chiasm, formed a rectangular block of tissue (200 mg) containing the hypothalamus and part of the overlying thalamus. Tissues were frozen on dry ice within 5 min after sacrifice and stored at −70 °C.

Cell Cultures. Cell Lines. C6 glioma, 2-B subclone (de Vellis and Brooker, 1973), and the B104 neuroblastoma were grown in medium consisting of 15% (v/v) fetal calf serum (Irvine Scientific; Irvine, Calif.) in Ham's F-10 (Gibco) without antibiotics. The cultures (Falcon flasks T250, 75 cm^2) were maintained in a humidified atmosphere of 5% CO_2 at 37 °C, with media changes every 2–3 days. Cells in a stationary phase (14 days incubation) were detached from the dishes using a calcium- and magnesium-free balanced salt solution containing 0.02% EDTA.[1] The cells were collected by centrifugation and frozen at −70 °C.

Primary Glial Cultures. Whole brains from newborn Wistar rat pups (1–2 days old) were used, after removal of meninges. The tissue was dissociated as described by McCarthy and de Vellis (1978) and grown in monolayer cultures. Cells were grown in basal Eagle's medium containing 15% fetal calf serum (Irvine Scientific) without antibiotics. Cultures were maintained under the same conditions and collected at 14 days (in stationary phase) by the same procedure as the cell lines.

Extraction of Total RNA. Frozen tissue or cells were homogenized in a Sorval Omnimixer (Sorval-DuPont; Newtown, Conn.) at 4 °C in 10 volumes (w/v) of 50 mM Tris-HCl (pH 7.4), 0.5% $NaDodSO_4$, 0.5% 1,5-naphthalenedisulfonate, 100 μg/mL poly(vinyl sulfate), and an equal volume of buffer-saturated phenol containing 0.1% 8-hydroxyquinoline (Davidson et al., 1964; Crippa et al., 1967). All subsequent organic extractions of RNA were carried out at 4 °C. The homogenate was shaken for 20 min, and the phases were separated by adding 1/8 volume of $CHCl_3$ and centrifuging in a Beckman SW-27 rotor (25 000 rpm/10 min). The aqueous phase was extracted twice more as above, and nucleic acids were precipitated by the addition of NH_4OAc and ethanol (Osterburg et al., 1975). The combined interphases were suspended in 0.1 M Tris-HCl (pH 8.6 at 20 °C), 0.5% $NaDodSO_4$, and 100 μg/mL poly(vinyl sulfate) (Hadjivassiliou and Brawerman, 1967) and extracted with buffer-saturated phenol, and the phases were separated as above. Under these conditions, 95% of the ethanol-precipitable radioactivity present in brain RNA pulse-labeled in vivo with [^{3}H]uridine was recovered from the interphase.

DNA in the extracts was eliminated prior to the isolation of poly(A) RNA. After additional NH_4OAc–ethanol precipitations, the sample was suspended in 0.1 M NH_4OAc, 10 mM $MgCl_2$, and 100 μg/mL poly(vinyl sulfate). DNase (DPFF, Worthington Biochemicals, Freehold, N.J.) previously purified from detectable RNase activity by iodoacetate treatment (Zimmerman and Sandeem, 1966) and exclusion chromatography on Sephadex G-50 (Smith et al., 1974) was added until a concentration of 100 μg/mL was reached and then incubated for 60 min at 0 °C. Preautodigested Pronase (Grade B; Sigma Chemical Co., St. Louis, Mo.) was added until a concentration of 50 μg/mL was reached, and the sample was incubated for an additional 30 min at 0 °C. The mixture was then brought to 1% $NaDodSO_4$, incubated for 60 min at 37 °C, extracted with $CHCl_3$–isoamyl alcohol (24:1, v/v), and precipitated with NH_4OAc–ethanol. DNA fragments were removed by resuspending the precipitate four times in 3.0 M NaOAc (Palmiter, 1973). The NaOAc-insoluble material was enriched in high-molecular-weight RNA (>95% of pulse-labeled RNA is retained) and contains small quantities of 4S and 5S RNA. The A_{260}/A_{280} and A_{260}/A_{230} ratios of all preparations were >2.0.

Isolation of Poly(A) RNA. Poly(A) RNA was isolated by binding to oligo(dT)-cellulose (Aviv and Leder, 1972). Total RNA in 10 mM Tris-HCl (pH 7.4), 0.5 M NaCl, and 0.5% $NaDodSO_4$ was applied to 1 g of packed cellulose (type 7, P. L. Biochemicals; Milwaukee, Wis.), and nonbound material was rechromatographed in the same buffer. The column was washed with the above buffer until the A_{260} of that eluate was at background. Bound poly(A) RNA was eluted with H_2O. The poly(A) RNA enriched fraction was rechromatographed to reduce ribosomal RNA contamination. About 50% of the RNA that bound to oligo(dT)-cellulose on the first pass failed to bind a second time. Further repassage of the sample reduced RNA binding by 10%. Brain poly(A) RNA is heterogeneous in size, with the majority sedimenting between 18 and 28 S on formaldehyde–sucrose gradients (Fenwick, 1968). The final preparation contained small amounts of rRNA (10–15%). Treatment of brain total RNA with dimethyl sulfoxide (80%, v/v) to reduce aggregation before passage over oligo(dT)-cellulose (Bantle et al., 1976) did not alter the poly(A) RNA yield or complexity. Therefore, we consider that contamination by poly(A) RNA "minus" sequences is not significant in this study.

Preparation of [^{3}H]DNA. In Vivo Labeled, Nonrepetitive [^{3}H]DNA. Total ^{3}H-labeled DNA was obtained from rat Walker carcinoma cells grown for 48 h with 5 μCi/mL [*methyl*-5-^{3}H]thymidine, (55 Ci/mmol, New England Nuclear; Boston, Mass.). DNA was isolated from purified nuclei

[1] Abbreviations used: C_0t, DNA concentration (mol of nucleotide/L) at t_0 × time (s); R_0t, RNA concentration at t_0 × time (s); [^{3}H]DNA, [^{3}H]thymidine-labeled DNA; HAP, hydroxylapatite; NH_4OAc, ammonium acetate; NaOAc, sodium acetate; nt, nucleotide; poly(A), polyadenylated; $NaDodSO_4$, sodium dodecyl sulfate; T_m, mean melting temperature; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

by the Marmur (1961) procedure with additional RNase and Pronase treatments and had a specific activity of 4.15×10^5 cpm/μg. DNA was sheared to 350 nucleotide fragments in the laboratory of E. H. Davidson by two passes through a needle valve at 50 000 psi (Britten et al., 1974). The sample was filtered through a Metricel GA-6 filter (Gelman Instrument Co.; Ann Arbor, Mich.) and passed over the column of AG50W-X2 (Bio-Rad Laboratories; Richmond, Calif.) overlayered on Chelex 100 (Smith et al., 1974). Unlabeled sheared DNA from purified rat liver nuclei was prepared similarly.

Care was taken to minimize contamination by moderately repetitive sequence elements. Total sheared ^{3}H-labeled DNA was denatured at 100 °C (5 min) and incubated in 0.41 M phosphate buffer at 60 °C to an equivalent C_0t of 1500. All C_0t values given here are corrected for the effect of [Na^+] on hybridization rate (Wetmur and Davidson, 1968). Unreassociated DNA was separated from duplex structures by chromatography on HAP (see below). The single-stranded fraction was reincubated to C_0t = 1000, and unreassociated DNA was isolated on HAP. The resulting [^{3}H]DNA tracer reassociated at C_0t values <50 to 2–3% with total rat nuclear DNA (Figure 1) and was, therefore, 30-fold enriched in nonrepetitive sequences. The fragment length was 287 ± 24 nucleotides (mean ± SEM), as determined by sedimentation on alkaline sucrose gradients (Studier, 1965).

In Vitro Labeled [^{3}H]DNA. [^{3}H]DNA was prepared from a nonrepetitive DNA template with *Escherichia coli* DNA polymerase I essentially as described by Galau et al. (1976). Unlabeled DNA from rat liver nuclei was isolated, purified, and sheared as above. Nonrepetitive DNA was then isolated by three cycles of reassociation to C_0t = 250 and separation of single-stranded DNA by HAP chromatography. The isolated nonrepetitive DNA was then reassociated to C_0t = 58 500 and used as a template. DNA (20 μg/mL) was incubated for 26 h at 10 °C in 0.05 M phosphate buffer (pH 7.6), 10 mM $MgCl_2$, 1 mM EDTA, containing 2×10^{-5} M each of dATP, dGTP, and dCTP and 1.89×10^{-5} M [^{3}H]dTTP (55 Ci/mmol, New England Nuclear) and 5 units of *E. coli* DNA polymerase I/μg of DNA (Boehringer Mannheim, grade I). The reaction was terminated by bringing the mixture to 0.12 M phosphate buffer and 25 mM EDTA, followed by digestion with Pronase (0.1 μg/mL) for 1 h at 37 °C and $CHCl_3$–isoamyl alcohol extraction. The [^{3}H]DNA, which bound to HAP in 0.12 M phosphate buffer at 60 °C, was eluted in the same buffer at 97 °C and rechromatographed on HAP to remove foldback complexes. The tracer was further purified on Sephadex G-100 and Chelex 100 in 0.24 M NH_4OAc, filtered (Metricel GA-6), and dialyzed against 0.05 M phosphate buffer. Its initial size was 350 nucleotides and the rate of reassociation to total sheared DNA was similar to the in vivo labeled probe. The final specific activity of the tracer was 6–7 $\times 10^6$ cpm/μg of DNA.

HAP Chromatography. Hybridization reaction mixtures were separated into single- and double-stranded fractions by chromatography on HAP (Britten and Kohne, 1968). To reduce nonspecific binding, HAP was heated at 100 °C for 20 min in 10 volumes of 0.12 M phosphate buffer (Britten et al., 1974).

Phosphate buffer (1 M PO_4, pH 6.8) was prepared from an equimolar mixture of mono- and dibasic sodium phosphate in deionized, distilled water, followed by passage over Chelex 100 (Davidson and Hough, 1971) and sterilization by Autoclaving. [PO_4^{3-}] was gravimetrically determined by precipitation with excess $AgNO_3$. The supernatant was brought to pH 7 with dilute NH_4OH, and the Ag_3PO_4 precipitate was filtered, washed with water and methanol, and dried. Based on the extreme insolubility of Ag_3PO_4, the recovery of PO_4 is >99.9%; the variation of multiple determinations was ≤0.5%.

Reaction mixtures diluted in 3 mL of 0.12 M phosphate buffer and 0.1% $NaDodSO_4$ were applied to 1 mL of packed HAP in jacketed columns at 60 °C equilibrated in the same buffer. Single strands were eluted with three 3-mL washes of 0.12 M phosphate buffer. Bound sequences were eluted as single strands in 0.12 M phosphate buffer at 97 °C or as intact duplexes with 0.48 M phosphate buffer at 60 °C. Irreversible binding of [^{3}H]DNA to HAP was determined by dissolving the HAP in 2 mL of 6 N HCl.

The T_m of DNA–DNA and RNA–DNA hybrids was obtained by thermal elution from HAP. After equilibration in 0.1 M phosphate buffer (Martinson and Wagenaar, 1974; Bantle and Hahn, 1976), duplexes were dissociated by raising the column temperature in 5 °C steps from 60 to 100 °C. After a 3-min equilibration at each temperature, nonbound material was eluted with 3 mL of 0.1 M phosphate buffer.

DNA Renaturation Reactions. Nonrepetitive [^{3}H]DNA was mixed with a 1700-fold excess of total sheared rat nuclear DNA in 0.41 M phosphate buffer, sealed in silanized (DMCS, Applied Science Labs; Inglewood, Calif.) glass capillary tubes, heat denatured (100 °C/5 min), and incubated at 60 °C. Reactions were terminated by dilution into 0.12 M phosphate buffer and rapid freezing in a solid CO_2–2-propanol bath. Duplex formation was determined on HAP by UV absorbance ($A_{260} - A_{320}$) and liquid scintillation counting in Aquasol (New England Nuclear).

RNA–DNA Hybridization Reactions. Nonrepetitive [^{3}H]DNA was mixed with poly(A) RNA in 0.41 M phosphate buffer, 1 mM EDTA, and 0.1% $NaDodSO_4$ (Galau et al., 1974), in RNA concentrations of 3–6 mg/mL and RNA/DNA ratios of 800–3500:1. Equivalent R_0t values calculated for each reaction were not corrected for the rRNA content (<15%) of the poly(A) RNA preparations. Aliquots of the reaction mixture (1–2 μL) were sealed in glass capillaries and denatured in boiling water for 5 min. After incubation at 70 °C for 0.5–120 h, reactions were terminated as above. DNA duplexes in RNA-driven reactions were estimated after digestion of RNA–DNA hybrids with RNase (Galau et al., 1974). After dilution of the reaction mixtures to 0.05 M phosphate buffer, RNase A was added until a concentration of 2 μg/mL was reached, and the mixtures were incubated at 37 °C for 24 h. RNase A was previously heated at 80 °C for 30 min to destroy contaminating DNase activity. RNase-resistant DNA duplexes were measured on HAP. Increasing the RNase concentration to 10 μg/mL did not alter the observed values of DNA duplexes, which ranged from 1.5 to 1.8% of the total [^{3}H]DNA input at R_0t values of 20 000–30 000.

Isolation of [^{3}H]DNA from RNA–DNA Hybrids. A mixture of nonrepetitive [^{3}H]DNA and whole brain poly(A) RNA was incubated at 70 °C to a R_0t of 32 800 at 5.3 mg/mL RNA and a RNA/DNA ratio of 800:1. The mixture was passed over HAP in 0.12 M phosphate buffer at 60 °C and duplex structures eluted with 0.48 M phosphate buffer at 60 °C. The eluate was diluted to 0.05 M phosphate buffer, and RNA–DNA hybrids were destroyed by RNase treatment as above. The sample was incubated at 37 °C for 60 min in the presence of Pronase (10 μg/mL), followed by $CHCl_3$ extraction. The mixture was adjusted to 0.03 M phosphate buffer, 0.1% $NaDodSO_4$ and applied to HAP equilibrated in the same buffer. Single-stranded DNAs from the RNA–DNA hybrids were recovered in 0.12 M phosphate buffer, mixed with sheared total rat nuclear DNA (driver), dialyzed against 0.24 M NH_4OAc, and precipitated with ethanol.

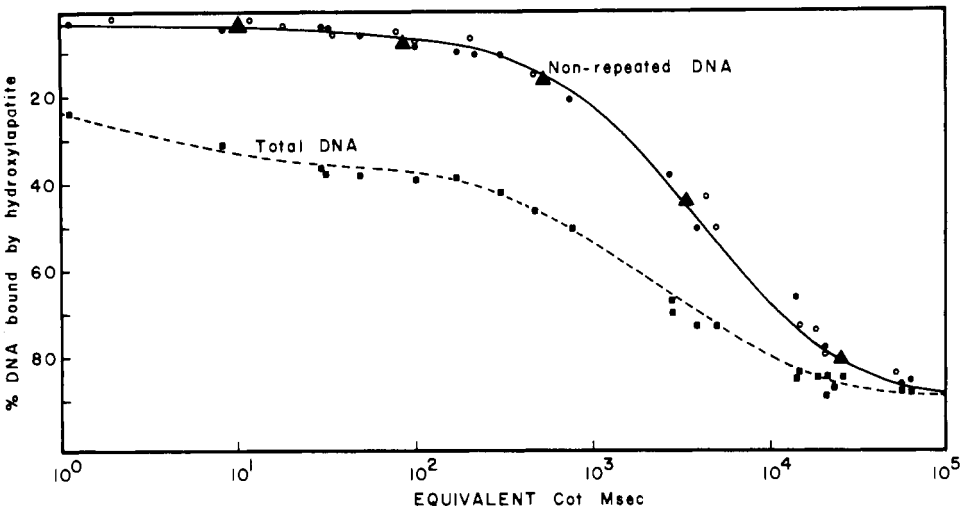

FIGURE 1: Reassociation kinetics of nonrepetitive [^{3}H]DNA (in vivo labeled) and unlabeled total DNA. Nonrepetitive [^{3}H]DNA (287 nucleotides) was renatured in the presence of excess total DNA (570 nucleotides) in 0.41 M phosphate buffer at 60 °C and DNA-DNA duplex formation measured by HAP column chromatography (see Materials and Methods): nonrepetitive [^{3}H]DNA from different preparations (●) (●,○); total sheared nuclear DNA (■); nonrepetitive DNA isolated from poly(A) RNA-DNA hybrids (▲). The curve describing the renaturation of nonrepetitive [^{3}H]DNA is a computer-determined, best least-squares fit with a root mean square error of 0.0172 and a second-order rate constant of 3.2×10^{-4} L mol^{-1} s^{-1} or a $C_0t_{1/2} = 3100$. The total DNA reassociated to 93% at $C_0t > 10^5$.

TABLE I: Binding of Nonrepetitive [^{3}H]DNA Tracer at HAP: "Zero Time" (Nonspecific) Binding and Irreversible Binding.

reaction mixture (60 °C)	C_0t (ms)	% duplex[a]	% irreversibly bound[b]
[^{3}H]DNA tracer + DNA			
total DNA (sheared)	<10	2.0	0.03
nonrepetitive DNA (sheared)	>25 000	80	0.10
	0.0005	0.3	0.20
reaction mixture (70 °C)	**R_0t (ms)**	**% duplex**	**% irreversibly bound**
[^{3}H]DNA tracer + RNA			
liver total RNA (0.003 mg/mL)	0.004	0.3	0.20
cortex poly(A) RNA (0.003 mg/mL)	0.004	0.6	0.20
whole brain poly(A) RNA (3.65 mg/mL)	0.015	1.0	0.05
	15 000	10.0	0.10

[a] Total duplex eluted from HAP with 0.12 M phosphate buffer at 97 °C. [b] Residual 3H-DNA not eluted with 0.48 M phosphate buffer (see Materials and Methods). [c] RNA/DNA ratios of 300:1 to 1000:1.

Analysis of Hybridization Kinetics. The kinetics of hybridization were calculated by a computer program for a best least-squares fit using iterative procedures according to pseudo-first-order kinetics for RNA-driven reactions (Bishop, 1972) and second-order kinetics for DNA reassociation (Britten and Kohne, 1968).

Results

Characterization of Nonrepetitive [^{3}H]DNA Tracer. Whereas approximately 30% of the total DNA reassociated rapidly, the nonrepetitive [^{3}H]DNA tracer (in vivo labeled) remained >97% single stranded at $C_0t < 50$ (Figure 1, Table I). Irreversible binding of the ^{3}H tracer to HAP was negligible (Table I). The nonrepetitive tracer reassociated with a second-order rate constant of 3.2×10^{-4} L mol^{-1} s^{-1} and reached a limit of 86% HAP bound duplexes. The [^{3}H]DNA remaining single stranded at $C_0t > 50\,000$ did not reassociate further when renatured in the presence of fresh total DNA. The in vivo and in vitro labeled nonrepetitive [^{3}H]DNA tracers behaved indistinguishably. Similar values for the proportion of nonrepetitive DNA and its reassociation rate constant were obtained for rat liver and ascites cell DNA (Holmes and Bonner, 1974; Pearson et al., 1978; Savage et al., 1978).

The reassociation rate constant of the in vivo labeled nonrepetitive [^{3}H]DNA predicts that the haploid genome contains 2.6×10^9 nucleotide pairs of nonrepetitive DNA. This calculation is made with reference to the *E. coli* genome incubated under identical conditions (Hough et al., 1975) and includes a fragment length correction (Wetmur and Davidson, 1968) for the 287 nucleotide tracer. This estimate of complexity is in reasonable agreement with that indicated for a haploid rat genome of $3\text{-}4 \times 10^{-12}$ g (Sober, 1968; Bachmann, 1972; Shapiro, 1976). Assuming that nonrepetitive DNA comprises 70% of the genome (Figure 1), its complexity would be in the range of $1.8\text{-}2.5 \times 10^9$ nucleotide pairs.

The T_m of nonrepetitive [^{3}H]DNA-total DNA hybrids was 82.6 °C (Figure 2). Under identical conditions, the T_m of sheared native DNA (570 nucleotides) was 87 °C. The in vivo labeled DNA tracer was used in the following studies, unless otherwise indicated.

Hybridization of Whole Brain Poly(A) RNA to Nonrepetitive [^{3}H]DNA. The formation of RNA-DNA hybrids during incubation is shown in Figure 3. The [^{3}H]DNA duplex content of the total hybrid fraction (see Materials and Methods) was independent of RNA-DNA ratios of 800-3000:1 and concentrations of 3-6 mg/mL. Since DNA duplex content did not exceed that predicted for the self-reassociation of the [^{3}H]-DNA tracer alone, the poly(A) RNA preparations contain

TABLE II: Yields and Complexity of Total Poly(A) RNA from Liver and Brain Regions.

tissue (prep no.)	total RNA (mg/g of tissue[a])	poly(A) RNA (μg/g of tissue)	obsd max hybridizat (saturat) value[b] (mean % ± SD) (n)[c]	T_m hybrids (°C)	corr max hybridizat value[d] (%)	complexity[e] (nt × 10^8)
liver (I, fresh)	5.33	11.5	5.2 ± 0.4 (8)	81.0	6.9	3.6
liver (II, frozen)	5.28	10.6	5.2 ± 0.5 (4)		6.9	
whole brain (I)	0.92	26.4	9.4 ± 1.7 (4)	80.3	12.5	6.7
whole brain (II)	0.85	26.4	9.6 ± 1.2 (6)	80.0	12.8	
cortex (I)	1.03	29.2	9.1 ± 1.3 (5)	80.0	12.1	6.5
cortex (II)	0.98	24.3	9.4 ± 2.3 (9)	79.5	12.5	
hypothalamus (I)	0.97	25.4	10.0 ± 1.2 (10)		13.3	6.9
hypothalamus (II)	1.02	23.4	9.7 ± 0.2[f] (6)	79.5	12.9	
hippocampus (I)	0.88	17.5	6.8 ± 1.1 (4)	80.5	9.1	5.4
hippocampus (II)	1.07	20.4	8.8 ± 1.2[f] (6)	80.0	11.7	
cerebellum	0.91	34.5	9.4 ± 1.0 (4)		12.5	6.6

[a] Yield by A_{260} of purified RNA before chromatography on oligo(dT)-cellulose. Because small RNA components are extracted by 3 M sodium acetate, total RNA content is probably underestimated by 10–20%. [b] Data obtained from hybridization reaction mixtures incubated to R_0t >15 000 ms at RNA/DNA ratios of 1000–3000:1. Corrected for [^{3}H]DNA duplex content. [c] n = number of data points analyzed. [d] Maximum value for [^{3}H]DNA reassociated in 0.41 M phosphate buffer at 70 °C in the presence of 1700-fold excess unlabeled sheared DNA was 75% at C_0t = 60 000. RNA/DNA hybridization values corrected to 100% [^{3}H]DNA reactivity. [e] Average value of both RNA preparations assumes that DNA transcription is asymmetric and that the rat nonrepetitive genome is 2.64 × 10^9 nucleotides (see Results). [f] There was no significant effect of the RNA/DNA ratio on values at R_0t of 25 000 for RNA–DNA hybrids: hypothalamus, 9.7 ± 0.10 (1000:1) and 9.8 ± 0.15 (3000:1); hippocampus, 8.8 ± 0.03 (1000:1) and 9.0 ± 0.01 (3000:1). The data for both ratios were, therefore, combined.

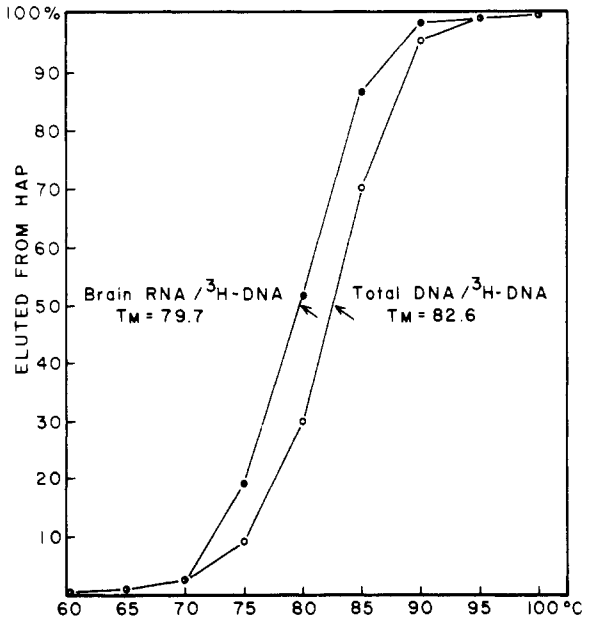

FIGURE 2: HAP thermal elution profile of RNA–DNA and DNA–DNA duplexes. Brain poly(A) RNA was incubated with nonrepetitive [^{3}H]DNA at 70 °C to a R_0t of 21 000 (3.5 mg of RNA/mL and a RNA/DNA ratio of 1000:1). Total sheared DNA was renatured in the presence of nonrepetitive [^{3}H]DNA at 70 °C.

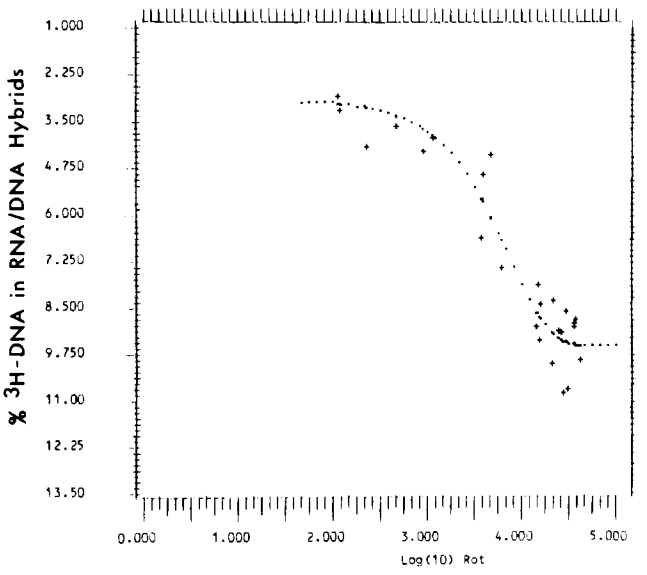

FIGURE 3: Kinetics of hybridization of nonrepetitive [^{3}H]DNA tracer to brain total poly(A) RNA. Semilogarithmic plot of data for RNA–DNA hybrids. Data from two RNA preparations with a computer graphed, best least-squares fit determined according to pseudo- first-order kinetics (see Materials and Methods). The pseudo-first-order rate constant of the reaction is 1.9 × 10^{-4} L mol^{-1} s^{-1}. Data are uncorrected for [^{3}H]DNA tracer reactivity.

minor DNA contamination. Interassay variation of maximum hybridization values from the same RNA preparation was 10–20%, with the greatest variations at high R_0t values. We attribute this variation to a decrease in the stability of the small-sized [^{3}H]DNA tracer that occurs during the prolonged incubations required to approach completion of hybridization with highly complex RNA.

The binding of [^{3}H]DNA to HAP after a short incubation with RNA (R_0t <0.1) was 0.3–1.0% (Table I). This estimates the upper limit of nonspecific or "zero-time" binding and probably also represents RNA–DNA hybrids formed from the abundant frequency classes of poly(A) RNA (Bantle and Hahn, 1976). Irreversible binding of [^{3}H]DNA to HAP was negligible (Table I).

The thermal elution profile of [^{3}H]DNA from HAP of hybrids formed with whole brain poly(A) RNA at R_0t > 15 000 (Figure 2) indicates well-matched RNA–DNA hybrids. The T_m of such hybrids was typically 80 °C (Table II) or 3 °C below that of nonrepetitive [^{3}H]DNA duplexes formed under identical conditions (Figure 2).

Since the nonrepetitive [^{3}H]DNA preparation may contain small but significant (2–3%) amounts of rapidly renaturing

DNA (Figure 1), the [^{3}H]DNA in the brain RNA-DNA hybrids was isolated (Material and Methods) and reassociated with a 1700-fold excess of sheared total DNA. The recycled [^{3}H]DNA (triangles in Figure 1) reassociated with a second-order rate constant of 2.8×10^{-4} L mol^{-1} s^{-1} and was kinetically indistinguishable from the starting nonrepetitive [^{3}H]DNA tracer. We conclude that the [^{3}H]DNA in RNA-DNA hybrids formed at high R_0t values is predominantly nonrepetitive DNA and can be used to estimate the nonrepetitive base sequence complexity of poly(A) RNA.

The hybridization of whole brain poly(A) RNA from two RNA preparations was analyzed according to pseudo-first-order kinetics (Bishop et al., 1974) by a computer program for best least-squares fit (Figure 3). A maximum of 9.5% of the [^{3}H]DNA formed RNA-DNA hybrids (Table II). Correcting for the fraction of the [^{3}H]DNA tracer which cannot form hybrids under identical hybridization conditions (Table II, footnote d), brain poly(A) RNA is homologous to 12.3% (average) of nonrepetitive DNA (Tables II and III). Assuming asymmetric transcription, the sequence complexity of whole rat brain poly(A) RNA is 6.5×10^8 nucleotides.

At least two major kinetic classes occur in brain total poly(A) RNA. After correcting for [^{3}H]DNA reactivity, the slowly hybridizing components were homologous to about 9% of the nonrepetitive [^{3}H]DNA (Figure 3) and hybridized with a pseudo-first-order rate constant of 1.9×10^{-4} L mol^{-1} s^{-1}. Rapidly hybridizing poly(A) RNA components were not studied in detail, but react with about 4% of the [^{3}H]DNA (corrected for [^{3}H]DNA reactivity) during incubations at R_0t values between 0.2 to 200. The relative amount of the [^{3}H]-DNA hybridizing to RNA at R_0t values < 200 clearly distinguished it from the 1% "zero-time" or nonspecifically bound [^{3}H]DNA (see above). Most of the complexity in slowly hybridizing poly(A) RNA probably represents heterogeneous nuclear poly(A) RNA (see Discussion).

The fraction of poly(A) RNA driving the slow phase of the hybridization reaction was calculated by comparing the observed reaction rate (K_{obsd}) with the rate expected (K_{exp}) if all the RNA consisted of complex sequences driving the reaction (Hough et al., 1975). Using the *E. coli* genome as a complexity standard (4.2×10^6 nucleotide pairs and K of 0.25 L mol^{-1} s^{-1}) to estimate K_{exp}, approximately 4% of the poly(A) RNA population derives the reaction. This calculation assumes that DNA-DNA and RNA-DNA nucleation rates are equal (Galau et al., 1977) and is corrected for differences in DNA fragment size (Wetmur and Davidson, 1968).

Complexity of Brain Region and Liver Poly(A) RNA. Total poly(A) RNA from the cerebral cortex, hypothalamus, and cerebellum yielded complexity values similar to that of whole brain (Table II). However, the complexity of hippocampal poly(A) RNA was less than that of the hypothalamus by 10–30% (Table II); in both preparations, the hippocampus and hypothalamus were processed and analyzed in parallel to minimize procedural variations. Although the interpreparation variance minimizes this possible regional difference, a smaller complexity of hippocampal poly(A) RNA would be consistent with its simpler histological structure and more limited number of differentiated cell types, as compared with the hypothalamus. The complexity of liver poly(A) RNA was 50–60% of that observed in whole brain or brain regions (Table II).

Cerebral cortical (Figure 4) and hypothalamic (data not shown) poly(A) RNA contained slowly hybridizing components in the same percentage (70%) as whole brain. The pseudo-first-order rate constants of these reactions were also indistinguishable from that of whole brain; cerebral cortex, 2.3 $\times 10^{-4}$ L mol^{-1} s^{-1} (Figure 4); hypothalamus, 2.0×10^{-4} L

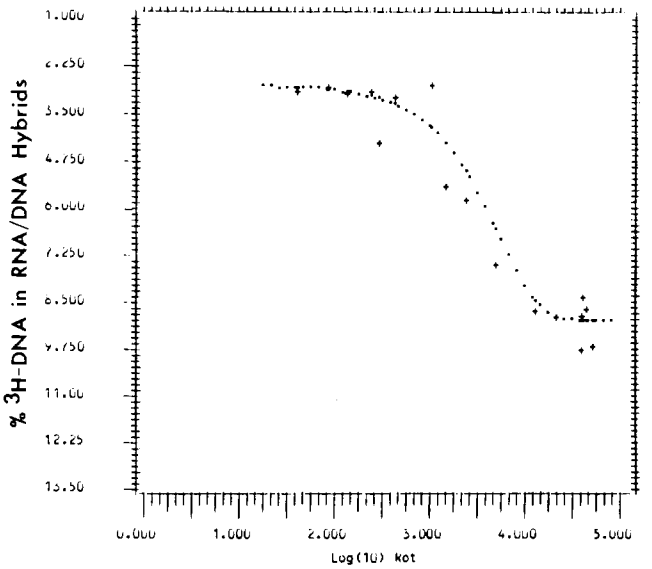

FIGURE 4: Kinetics of hybridization of nonrepetitive [^{3}H]DNA tracer to cerebral cortex poly(A) RNA. Semilogarithmic plot of data for RNA-DNA hybrids from two RNA preparations with a computer graphed, best least-squares fit determined according to pseudo-first-order kinetics (see Materials and Methods). The pseudo-first-order rate constant of the reaction is 2.3×10^{-4} L mol^{-1} s^{-1}. Data are uncorrected for [^{3}H]DNA tracer reactivity.

mol^{-1} s^{-1} (data not shown). Although an insufficient quantity of hippocampal RNA prevented detailed kinetic studies, the RNA-DNA hybrids formed at R_0t values of <100 were 3.8 ± 0.5%, a value similar to that obtained with hypothalamus poly(A) RNA (3.7 ± 0.2%).

Hybridization Studies with Total Poly(A) RNA from Cultured Cells of Neural Origin. The extent to which neurons and glia contribute to the transcriptional diversity of brain was approached indirectly using cultured cells of neural origin. In vitro, rat B104 neuroblastoma retains several properties of differentiated neurons, e.g., an excitable membrane and extension of neurites, acetylcholine receptors, and the neuron-specific 14-3-2 protein (Shubert et al., 1974). Under the present culture conditions, the 2B subclone of C6 glioma (de Vellis and Brooker, 1977) manifests several characteristics of glia in vivo such as abundant S-100 protein (Benda et al., 1968), the glial fibrillary acidic protein (Bissell et al., 1974), 2′,3′-cAMP 3′-phosphohydrolase (Zanetta et al., 1972), and the glucocorticoid-inducible glycerophosphate dehydrogenase, an oligodendrocyte marker (de Vellis and Brooker, 1973; McGinnis and de Vellis, 1974). Primary glial cultures from 3-day-old rat brain consisted of 80% astrocytes and 20% oligodendroglia (McCarthy and de Vellis, 1978).

Cultured rat cells in stationary phase had total poly(A) RNA which hybridized to in vitro labeled nonrepetitive [^{3}H]DNA to a smaller extent than with brain ($p < 0.001$) and higher than with liver poly(A) RNA ($p < 0.02$) in the order: liver < primary glial cultures < neuroblastoma < glioma < brain (Table III). The hybridization of combinations of RNA from these sources (in equal amounts) to R_0t values of 2.0–3.5 $\times 10^4$ yielded values which were 89–98% of the highest cell type or organ in the combination. Because each RNA sample in these experiments was present at one-half the concentration as when hybridized alone, data from combination experiments represent minimum hybridization values. Taken together, however, these results indicate extensive similarities in total poly(A) RNA sequences between neuroblastoma, glioma, and glial cells and suggest that most liver total poly(A) RNA se-

TABLE III: Hybridization of Total Poly(A) RNA from Cultured Cells of Neural Origin (Separately and in Combinations Including Brain and Liver).

poly(A) RNA source and combination	R_0t (ms)	% [^{3}H]DNA hybridized[a] (mean ± SD) (no. of samples)
brain[b]	23 600	11.6 ± 0.4 (4)
brain + liver	32 800	11.0 ± 0.5 (3)
brain + neuroblastoma	25 800	11.0 ± 0.3 (4)
neuroblastoma[c]	26 600	8.7 ± 0.4 (4)
neuroblastoma + glioma[d]	21 900	8.4 ± 0.6 (3)
neuroblastoma + glial	26 100	8.0 ± 0.1 (3)
neuroblastoma + liver	29 200	7.7 ± 0.2 (3)
glioma	31 000	9.3 ± 0.2 (4)
glioma + liver	29 350	8.5 ± 0.1 (4)
glial	24 900	7.1 ± 0.3 (4)
liver	29 200	6.5 ± 0.1 (4)

[a] Poly(A) RNA was hybridized with nonrepetitive [^{3}H]DNA (in vitro labeled) at 3.5–6.0 mg of RNA/mL (RNA/DNA ratios of 3000:1) for 96 h. Combinations contained equal amounts of RNA from each source. Each sample was analyzed for content of total hybrids and DNA duplexes. RNA–DNA hybrids, corrected for [^{3}H]-DNA reactivity (81%). [b] From 2-month old Fischer 344 rats. [c] B104 rat neuroblastoma. [d] C6 rat glioma; 2B subclone.

quences are subsets of those in neuroblastoma, glioma, and brain.

Discussion

Characterization of Hybridization Reactions. The sequence complexity of poly(A) RNA was determined by RNA-driven hybridization reactions with nonrepetitive [^{3}H]DNA. The maximum hybridization of poly(A) RNA from adult rat brain to nonrepetitive DNA was 11.6–12.8%, values similar to the 13.3% reported for the adult mouse brain (Bantle and Hahn, 1976). The hybridization of brain RNA to nonrepetitive [^{3}H]DNA indicates at least two kinetically distinct components (Figures 3 and 4), designated as rapidly and slowly hybridizing classes of poly(A) RNA. As seen in Table I, the rapidly hybridizing components are clearly distinguished from nonspecific or "zero time" binding. Since the complexities of brain nuclear poly(A) RNA and whole brain poly(A) RNA are identical and are 3.5 times greater than polysomal poly(A) mRNA (Bantle and Hahn, 1976), the slowly hybridizing components are predominantly heterogeneous nuclear poly(A) RNA.

The complexities of total poly(A) RNA and the slowly hybridizing components can be calculated from the extent of their reaction with nonrepetitive [^{3}H]DNA, since the second-order rate constant of the [^{3}H]DNA isolated from RNA–DNA hybrids was indistinguishable from that of the initial nonrepetitive [^{3}H]DNA tracer. The complexity of rat brain total poly(A) RNA, 6.5×10^8 nucleotides, is somewhat higher than the 4.8×10^8 nucleotides obtained by Bantle and Hahn (1976) from mouse brain poly(A) RNA. This apparent discrepancy results from the use of a smaller value for the complexity of the mouse genome by those authors.

The fraction of poly(A) RNA driving the reaction of the slowly hybridizing components, calculated from the ratio of the rate expected from the observed complexity at saturation to its pseudo-first-order reaction rate constant (Hough et al., 1975), was approximately 4% of the poly(A) RNA mass for whole brain or brain regions. This value is similar to those reported for total nuclear or poly(A) nuclear RNA from mouse brain (Bantle and Hahn, 1976; Chikaraishi et al., 1978), Friend erythroleukemia cells (Kleiman et al., 1977), and sea urchin gastrulas (Hough et al., 1975).

Brain Region RNA. The similar complexities of poly(A) RNA from the hypothalamus, cerebral cortex, cerebellum, hippocampus, and whole brain suggest extensive similarities in the poly(A) RNA sequences of various rat brain regions. Additionally, the similar hybridization kinetics of poly(A) RNA from whole brain, cerebral cortex, or hypothalamus suggest no major regional differences in the complexities of the rapidly and slowly hybridizing RNA fractions. We suggest that variations in complexity [e.g., as observed between different RNA preparations for whole brain, liver (Tables II and III), and brain regions (Table II)] may obscure regional differences in complexity which are <10–20%. Thus, these studies do not eliminate the possibility that significant fractions of the total poly(A) RNA sequences are region specific. The general similarity in poly(A) RNA complexity from brain regions differing greatly in structure and function is in accord with a previous study of brain region RNA (Hahn, 1973) and is consistent with the paucity of reported brain region-specific proteins (e.g., Davies, 1970; Margolis, 1972; Vaughan and Calvin, 1977).

It is of interest that the complexity of liver total poly(A) RNA is about 50% of that in whole brain or brain regions. This difference, which is similar in proportion to the complexity differences in polysomal poly(A) mRNA (Young et al., 1976) and nuclear RNA (Chikaraishi et al., 1978) between liver and brain, indicates major tissue differences in the complexity of nuclear poly(A) RNA.

The Frequency of Different RNA Sequences in Brain Cells. The small fraction of the complex poly(A) RNA driving the slow phase of the RNA–DNA hybridization reaction (Figures 3 and 4) leads to an interesting prediction concerning the distribution of poly(A) RNA sequences in brain cells. The cellular levels of complex poly(A) RNA can be estimated from the yield of poly(A) RNA (Table II, 26 μg/g of brain) and fraction of RNA driving the reaction (0.04). Assuming these RNAs are randomly distributed and that the adult brain contains about 0.9×10^8 cells/g (Grouse et al., 1978), there are 1.16×10^{-14} g of complex poly(A) RNA per cell ($26 \times 10^{-6} \times 0.04/0.9 \times 10^8$); this is equivalent to 21.3×10^6 nt/cell (1.16×10^{-14} g/0.55×10^{-21} g/nt). Dividing the amount of complex poly(A) RNA per cell (21.3×10^6 nt/cell) by the complexity of brain total poly(A) RNA (6.7×10^8 nt, Table II), we calculate that each brain cell contains an average of 0.03 copy of each different complex poly(A) RNA sequence. This implies that approximately one copy of each different complex poly(A) RNA is present per 30 cells or that there are at least 30 different brain cell classes distinguished by their content of different complex poly(A) RNA sequences. The cerebellum may represent a more extreme case, because its cell density is three to five times that of other brain regions (McEwen et al., 1972), whereas the yield and complexity of its total poly(A) RNA are similar to other regions (Table II). The number of different complex class poly(A) RNA sequences per cell in brain regions and whole brain is one-tenth or less of that calculated for complex classes of RNA in other differentiated tissues (Hough et al., 1975; Ryffel and McCarthy, 1975; Axel et al., 1976) or cultured cells (Bishop et al., 1974; Ryffel and McCarthy, 1975; Kleiman et al., 1977). In contrast to sparsely distributed brain poly(A) RNA sequences, it can be calculated from the hybridization kinetics and complexity of brain nuclear RNA (Bantle and Hahn, 1976; Chikaraishi et al., 1978; Grouse et al., 1978) that there is also a class of hnRNA se-

quences present in much higher frequencies per cell. The extent to which the abundant and sparsely distributed classes of RNA contain brain-specific transcripts is presently unknown.

RNA from Cultured Neural Cells. The total poly(A) RNA complexity of primary glial cultures, B104 neuroblastoma, and C6 glioma was 10–35% greater than liver but less than that of brain (Table III). Hybridization of RNA from these sources indicates that neuroblastoma, glioma, and glial cells contain very similar poly(A) RNA sequences and that most liver and neuroblastoma poly(A) RNA sequences are subsets of those in brain. Similar studies with total nuclear RNA also indicated that the great majority of rat liver nuclear RNA sequences are also present in brain (Chikaraishi et al., 1978).

The total poly(A) RNA complexity of the cultured neural cells may be greater than for other types of cultured cells. For example, the maximum hybridization of nuclear poly(A) RNA with nonrepetitive DNA was 5.9% for mouse Friend erythroleukemia cells (Kleiman et al., 1977) and 2.5% for mouse embryonal carcinoma cells (Jacquet et al., 1978). By comparison, our hybridization values for cultured neural cells were 7.1–9.3%.

Our results, which indicate that RNA complexity of cultured neural cells is close to that of the brain, differ significantly from an early study of brain tumor cells (Grouse et al., 1973) and raise the possibility that neural cells of diverse origin, whether neurones or glia, have RNA populations of greater complexity than most cell types. These inferences should be regarded as provisional, since the RNA sequence content of the nucleus is different than that in the cytoplasm and, in some cases, may not show a corresponding extent of histospecific complexity (Kleene and Humphreys, 1977; Wold et al., 1978). Taken together, the available data suggest the possibility that the similarity of poly(A) RNA in different brain regions results, in part, from their content of neuronal and glial cell types, each of which has a high "constitutive" RNA complexity.

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References

Aviv, H., and Leder, P. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 1408–1412.

Axel, R., Feigelson, P., and Schutz, G. (1976), *Cell 7,* 247–252.

Bachmann, K. (1972), *Chromosoma 37,* 85–93.

Bantle, J. A., and Hahn, W. E. (1976), *Cell 8,* 139–150.

Bantle, J. A., Maxwell, I. H., and Hahn, W. E. (1976), *Anal. Biochem. 72,* 413–427.

Benda, P., Lightbody, J., Sato, G., Levine, L., and Sweet, W. (1968), *Science 161,* 370–371.

Bishop, J. O. (1972), *Acta Endocrinol. (Copenhagen), Suppl. 168,* 247–277.

Bishop, J. O., Morton, J. C., Rosbash, M., and Richardson, M. (1974), *Nature (London) 250,* 199–204.

Bissell, M., Rubinstein, L., Rignami, A., and Herman, M. (1974), *Brain Res. 82,* 77–89.

Brawerman, G., Gold, L., and Eisenstadt, J. (1963), *Proc. Natl. Acad. Sci. U.S.A. 50,* 630–638.

Britten R. J., and Kohne, D. E. (1968), *Science 161,* 529–540.

Britten, R. J., Graham, D. E., and Neufeld, B. R. (1974), *Methods Enzymol. 29E,* 363–406.

Brown, I. R., and Church, R. B. (1971), *Biochem. Biophys. Res. Commun. 42,* 850–856.

Chikaraishi, D. M., Deeb, S. S., and Sueoka, N. (1978), *Cell 13,* 111–120.

Crippa, M., Davidson, E. H., and Mirsky, A. E. (1967), *Proc. Natl. Acad. Sci. U.S.A. 57,* 885–890.

Davidson, E. H., and Hough, B. R. (1971), *J. Mol. Biol. 56,* 491–506.

Davidson, E. H., Allfrey, V. G., and Mirsky, A. E. (1964), *Proc. Natl. Acad. Sci. U.S.A. 52,* 501–508.

Davies, W. E. (1970), *J. Neurochem. 17,* 297–303.

de Vellis, J., and Brooker, G. (1973), in Tissue Culture of the Nervous System (Sato, G., Ed.) pp 231–245, Plenum Press, New York, N.Y.

de Vellis, J., McGinnis, J. F., Breen, G. A. M., Leveille, P., Bennett, K., and McCarthy, K. (1977), in Cell, Tissue and Organ Cultures in Neurobiology (Fedoroff, S., and Hertz, L., Eds.) pp 485–511, Academic Press, New York, N.Y.

Fenwick, M. L. (1968), *Biochem. J. 197,* 851–859.

Galau, G. A., Britten, R. J., and Davidson, E. H. (1974), *Cell 2,* 9–21.

Galau, G. A., Klein, W. H., Davis, M. M., Wold, B. J., Britten, R. J. and Davidson, E. H. (1976), *Cell 7,* 487–505.

Galau, G. A., Britten, R. J., and Davidson, E. H. (1977), *Proc. Natl. Acad. Sci. U.S.A. 74,* 1020–1023.

Grouse, L., Chilton, M. D., and McCarthy, B. J. (1972), *Biochemistry 11,* 798–805.

Grouse, L., Omenn, G. A., and McCarthy, B. J. (1973), *J. Neurochem. 20,* 1063–1073.

Grouse, L. D., Schrier, B. K., Bennett, E. L., Rosenzweig, M. R., and Nelson, P. G. (1978), *J. Neurochem. 30,* 191–203.

Hadjivassiliou, A., and Brawerman, G. (1967), *Biochemistry 6,* 1934–1941.

Hahn, W. E. (1973), *Annu. Meet. Soc. Neurosci. 3,* 139.

Hahn, W. E., and Laird, C. D. (1971), *Science 173,* 158–160.

Hough, B. R., Smith, M. J., Britten, R. J., and Davidson, E. H. (1975), *Cell 5,* 291–299.

Jacquet, M., Affara, N. A., Robert, B., Jakob, H., Jacob, F., and Gros, F. (1978), *Biochemistry 17,* 69–79.

Kleene, K. C., and Humphreys, T. (1977), *Cell 12,* 143–155.

Kleiman, L., Birnie, G. D., Young, B. D., and Paul, J. (1977), *Biochemistry 16,* 1218–1223.

Margolis, F. L. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 1221–1224.

Marmur, J. (1961), *J. Mol. Biol. 3,* 208–218.

Martinson, H. G., and Wagenaar, E. B. (1974), *Anal. Biochem. 61,* 144–154.

McCarthy, K. D., and de Vellis, J. (1978), *J. Cyclic Nucleotide Res. 4,* 15–26.

McEwen, B. S., Plapinger, L., Wallach, G., and Magnus, C. (1972), *J. Neurochem. 19,* 1159–1170.

McGinnis, J. F., and de Vellis, J. (1974), *Nature (London) 250,* 422–424.

Osterburg, H. H., Allen, J. K., and Finch, C. E. (1975), *Biochem. J. 147,* 367–368.

Palmiter, R. D. (1973), *J. Biol. Chem. 248,* 2095-2106.
Pearson, R., and Pearson, L. (1976), The Vertebrate Brain, Academic Press, New York, N.Y.
Pearson, W. R., Wu, J. R., and Bonner, J. (1978), *Biochemistry 17,* 51-59.
Ryffel, G. U., and McCarthy, B. J. (1975), *Biochemistry 14,* 1379-1384.
Savage, M. J., Sala-Trepat, J. M., and Bonner, J. (1978), *Biochemistry 17,* 462-467.
Schubert, D., Heineman, S., Carlisle, W., Tarikas, H., Kimes, B., Patrick, J., Steinbach, H. J., Culp, W., and Brandt, B. L. (1974), *Nature (London) 249,* 224-227.
Shapiro, H. S. (1976) in Cell Biology, (Altman, P. L., and Katz, D. D., Eds.) p 370, Federation of American Societies for Experimental Biology, Bethesda, Md.
Smith, M. J., Hough, B. R., Chamberlin, M. E., and Davidson, E. H. (1974), *J. Mol. Biol. 85,* 103-126.
Sober, H. A. (1968), Handbook of Biochemistry, Chemical Rubber Co., Cleveland, Ohio.
Studier, F. W. (1965), *J. Mol. Biol. 11,* 373-390
Vaughan, W. J., and Calvin, M. (1977), *Gerontology 23,* 110-126.
Wetmur, J. G., and Davidson, N. (1968), *J. Mol. Biol. 31,* 349-370.
Wold, B. J., Klein, W. H., Hough-Evans, B. R., Britten, R. J., and Davidson, E. H. (1978), *Cell 14,* 941-950.
Young, B. D., Birnie, G. D., and Paul, J. (1976), *Biochemistry 15,* 2823-2829.
Zanetta, J., Benda, P., Gombos, G., and Morgan, I. (1972), *J. Neurochem. 19,* 881-883.
Zimmerman, S. B., and Sandeem, G. (1966), *Anal. Biochem. 14,* 269-277.

Structure of the Chromatosome, a Chromatin Particle Containing 160 Base Pairs of DNA and All the Histones†

Robert T. Simpson

ABSTRACT: I have prepared chromatin particles from chicken erythrocytes which contain a 160 base pair length of DNA, an octamer of the four smaller histones, and a molecule of lysine rich histone, H1 or H5, and have compared some physicochemical properties of these particles with those of core particles, which lack H1 or H5 and contain 20 base pairs less of DNA. The former particles, called chromatosomes for convenience, are of particular interest in that they contain all the major components necessary for organization of the structure of long stretches of chromatin. Assuming that the structure of the 140 base pairs of DNA common to the core particle and the chromatosome is similar in these two particles, physicochemical data suggest that (1) the additional DNA in the chromatosome is folded around the histone core, (2) the additional DNA is not in the same conformation as the 140 base pairs of DNA in the core particle, and (3) both the 20 additional base pairs of DNA in the chromatosome and some DNA segments of the core particle itself are markedly stabilized to thermal denaturation, presumably by interaction with the lysine rich histone. Using 5′-end labeling, I have mapped the relative susceptibilities of DNase I cleavage sites in the chromatosomes. Sites at 20, 50, 60, and 100 bases from the ends are highly susceptible; the sites 30, 40, and 70-90 bases from the ends are cleaved with lower frequency. These data suggest that the chromatosome likely contains two full turns of DNA around the histone nucleus of the core particle, with a molecule of lysine rich histone bound outside the DNA-inner histone complex.

When chromatin is degraded by micrococcal nuclease, there are several levels of structural organization from which nucleoprotein particles can be isolated and studied with regards to their composition and structure; all of these are metastable intermediates in the course of a hydrolytic reaction which leads from the intact, high molecular weight DNA of the cell nucleus to the limit digest, at which point about half of the DNA has been degraded to acid solubility. The major pauses in the degradative process occur at: (1) a 40S particle consisting of about eight nucleosomes in a compact structure (Hozier et al., 1977); (2) the nucleosome, a particle containing the biochemical repeat length of DNA in the particular tissue, usually 185-205 base pairs (bp) of DNA (Noll, 1974; Axel, 1975; Sollner-Webb & Felsenfeld, 1975; Shaw et al., 1976; Noll & Kornberg, 1977); (3) a particle, which, for convenience, I will call a chromatosome, containing about 160 bp of DNA, one molecule of H1 (or H5), and an octamer of the four smaller histones (Varshavsky et al., 1976; Whitlock & Simpson, 1976a; Bakayev et al., 1977; Todd & Garrard, 1977; Noll & Kornberg, 1977); and (4) the core particle, consisting of 140 bp of DNA and the inner histone octamer, but lacking any lysine rich histone. The last of these species has been subjected to intensive physicochemical characterization, leading to a fairly detailed understanding of its structure (for reviews, see Kornberg, 1977; Felsenfeld, 1978). In progressing from our current understanding of the core particle towards an eventual understanding of the overall organization of chromatin, it seems that the structure of the chromatosome might be of particular interest, since this particle contains all the major components necessary for the structural organization of long stretches of chromatin, all the histones and DNA. While the structure and role of the apparently variable length of linker DNA which connects such particles remain uncertain, it should be noted that certain species have biochemical repeat lengths of near 160 bp of DNA (Morris, 1976; Noll, 1976; Lohr et al.,

† From the Developmental Biochemistry Section, Laboratory of Nutrition and Endocrinology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014. *Received July 19, 1978.*