labeled for 30 min and 2 hr these could be caused by a decrease with time in the size of the poly(A) segment in the RNA molecules. They could also be due to synthesis in the nucleus of smaller poly(A) segments after the cells have been incubated for an extended period of time *in vitro*, probably under less than optimal conditions.

It remains to be determined whether all species of mammalian mRNA carry the poly(A) segment. The polysomes also contain rapidly labeled non-rRNA that lacks poly(A). This RNA appears to reach the polysomes immediately after its synthesis is completed, and it does not seem to be affected by cordycepin. Part of this RNA shows sedimentation characteristics expected of mRNA. The exact relation of this RNA to the polysomes, however, remains to be determined.

Acknowledgments

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Hybridization of Ribonucleic Acid with Unique Sequences of Mouse Deoxyribonucleic Acid†

Larry Grouse,* Mary-Dell Chilton, and Brian J. McCarthy

ABSTRACT: The proportion of the genome transcribed in various mouse organs was estimated by hybridizing purified unique sequences of DNA with excess RNA. Saturation hybridization values of 4–5% were obtained for liver, kidney, and spleen, but as much as 11% for mouse brain. Recycling and additivity tests demonstrated a partial overlap in base sequences transcribed in various organs. The saturation value increased with age for brain RNA but little effect of age was apparent with liver RNA. Parallel experiments with *Bacillus subtilis* nucleic acids revealed that a relatively larger per cent of the bacterial genome is transcribed in exponentially growing bacteria.

For all the rapid increase in factual knowledge which has accumulated during recent years, the details of mechanisms controlling selective gene expression in mammalian cells remain obscure. For example, although the existence of a rapid turnover of nuclear RNA in mammalian cells has been established since the early work of Harris (1963), the relationship of this phenomenon to regulation of gene function is not yet understood. Since the size of the mammalian genome is so large compared to that of bacteria, it might be supposed that the fraction of the genome being transcribed into mRNA molecules at a given instant is quite small. Certainly a few per cent of the mammalian genome would suffice to specify essentially all known enzymes. Notwithstanding the appeal of such an argument, little quantitative data exist concerning the fraction of active genes in any mammalian cell or tissue.

An obvious approach to this question involves the use of DNA–RNA hybridization. In principle, the DNA of a cell may be titrated by total RNA to provide an estimate of the fractional transcriptional activity. Only those DNA segments which produced RNA in that cell will react. In practice, however, the assay is a difficult one for many reasons. In the first place, saturation of the DNA is all but impossible to achieve in cases where the frequency of various RNA molecules in a

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TABLE I: Single-Strand Molecular Weight of DNA Fragments Produced by Shearing[a] or by Depurination and Alkali Cleavage.[b]

DNA Treatment	$s_{20,w}$	Mol Wt, $\times 10^5$
11-min depurination at 70°	10.15	2.8
33-min depurination at 70°	7.83	1.7
66-min depurination at 60°	9.03	2.2
120-min depurination at 50°	14.7	5.4
Sheared at 10,000 psi	13.0	4.3

[a] Sheared DNA (see Materials and Methods) was denatured at pH 13 (22°), neutralized with NaH_2PO_4 solution and dialyzed *vs.* 1 M NaCl–0.5 M sodium citrate for this determination. [b] Depurinated DNA after alkali cleavage and neutralization (see Materials and Methods) was dialyzed *vs.* 1 M NaCl–0.05 M sodium citrate for this determination.

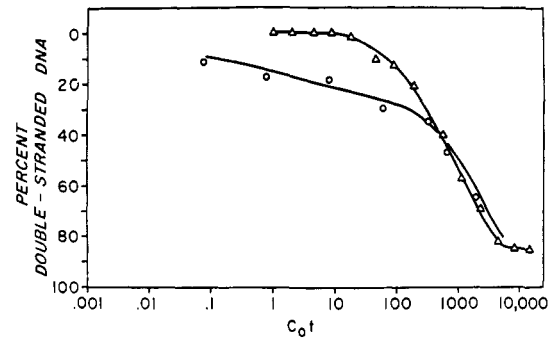

FIGURE 1: Renaturation kinetics of purified unique DNA and total mouse DNA. Labeled unique 33-min depurinated (Δ) and labeled total mouse DNA (○) were diluted 1000-fold with unlabeled 33-min depurinated total mouse DNA, adjusted to 48% formamide, 5 × SSC, denatured at 1000° and allowed to renature at 37°. Aliquots were assayed by hydroxylapatite chromatography as described in Materials and Methods. Data are corrected for hydroxylapatite background.

population is grossly different. Even for bacteria estimates of the fraction of transcribed genes have been in some disagreement varying from essentially 100% (McCarthy and Bolton, 1964) to about 50% (Kennell, 1968). In addition, mammalian genomes contain sets of base sequences which although not identical are so similar as to be operationally redundant (Britten and Kohne, 1968). In this case, RNA molecules transcribed from one DNA segment may react with another DNA sequence. This effect complicates the interpretation of saturation experiments with unfractionated mammalian DNA (Church and McCarthy, 1968; McCarthy and Church, 1970).

Although this problem can be minimized through the use of highly specific hybridization reaction conditions (Church and McCarthy, 1968), saturation hybridization experiments are most readily performed with DNA fractionated to remove those sequences which appear redundant. When this is accomplished, saturation hybridization experiments can be performed which yield estimates of the fraction of active genes in the unique sequence portion of the DNA expressed in cells of various tissues. In the present paper, we present some preliminary estimates of this fraction for liver, spleen, kidney, and brain of young adult mice. Values of only a few per cent were obtained in each case, except that the fraction of active genes appears to be much higher in the brain. Similar findings have been recently reported by Hahn and Laird (1971) and Brown and Church (1971).

Materials and Methods

Preparation of ^{3}H-Labeled DNA. Labeled mouse DNA was isolated from mouse L cells grown in monolayers in Eagle's medium containing [^{3}H]thymidine according to the method described by Hoyer *et al.* (1964). Labeled *Bacillus subtilis* DNA was isolated by a modification of Marmur's (1961) procedure from strain WB 746 grown in the presence of [6-^{3}H]-uracil as previously described (Chilton and Hall, 1968).

Degradation of DNA by Depurination and Alkali Cleavage. Partial depurination was carried out in 0.1 M acetate buffer (pH 4.2) at 70° unless otherwise stated (McConaughy and McCarthy, 1967). The reaction was terminated by cooling the solution to 0°. Per milliliter of reaction mixture, 0.2 ml of 1 M NaOH was added; the solution was heated at 50° for 10 min and cooled to 0°; neutralization was effected with 0.15 ml of 1 M NaH_2PO_4/ml of previous solution. DNA fragments were dialyzed against 0.01 × SSC and stored in a freezer.

Degradation of DNA by Shearing. DNA solutions in 0.1 × SSC were sheared in a pressure cell at 12,000 psi as previously described (McCarthy and Bolton, 1964). Sheared DNA solutions were stored in a freezer.

Determination of Molecular Weight of DNA Fragments. Sedimentation velocity measurements were made in a Spinco Model E ultracentrifuge. DNA concentration was *ca.* 40 μg/ml. Correction to $s_{20,w}$ and calculations of molecular weight were made as described by Studier (1965) (see Table I).

Isolation of Unique Sequences of ^{3}H-Labeled L-Cell DNA. ^{3}H-Labeled L-cell DNA (120 mg) which had been depurinated for 33 min was renatured to C_0t = 200 in 48% formamide, 5 × SSC at 37°. The DNA was separated into single-stranded and double-stranded fractions on a 2.0-ml column of hydroxylapatite (Clarkson) at 60°. The single-stranded fraction was isolated by elution with 0.12 M phosphate buffer while the double-stranded fraction was isolated by elution with 0.5 M phosphate buffer. The single-stranded fraction was passed over a second hydroxylapatite column to assure complete removal of all double-stranded material from the unique sequences. Earlier experiments with unique sequences purified by a single passage over hydroxylapatite indicated that they were contaminated with about 5% redundant sequences.

Figure 1 shows the renaturation kinetics of purified "unique" sequences used for subsequent experiments. Slight contamination of "unique" by "redundant" sequences cannot be ruled out by these data. However, other evidence will be presented on this point later.

Renaturation Kinetics for "Unique" Sequences. Labeled "unique" mouse DNA was diluted 1000-fold with unlabeled 33-min depurinated total mouse DNA, adjusted to 48% formamide, 5 × SSC, denatured at 100° and allowed to renature at 37°. Aliquots were assayed by hydroxylapatite chromatography as described in Materials and Methods.

Preparation of RNA. Total mouse tissue RNA was prepared by hot phenol extraction of homogenized tissue in the presence of sodium dodecyl sulfate at pH 5.2, removal of phenol by Sephadex gel filtration, treatment with electrophoretically pure DNase, phenol extraction, and precipitation with ethanol (Shearer and McCarthy, 1967). All RNA preparations were passed over Sephadex G-50 prior to hybridization experiments.

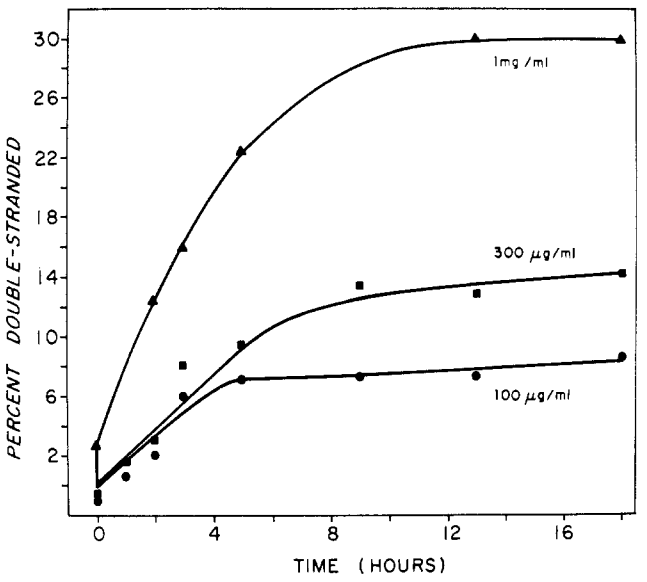

FIGURE 2: Hybridization of *B. subtilis* RNA with labeled DNA. *B. subtilis* DNA (0.2 μg/ml) was hybridized with *B. subtilis* RNA at 100 μg/ml (●), 300 μg/ml (■), and 1 mg/ml (▲) in 48% formamide, 5 × SSC; heated at 100° for 5 min immediately before incubating at 37° for hybridization kinetic studies, and aliquots were assayed by hydroxylapatite chromatography.

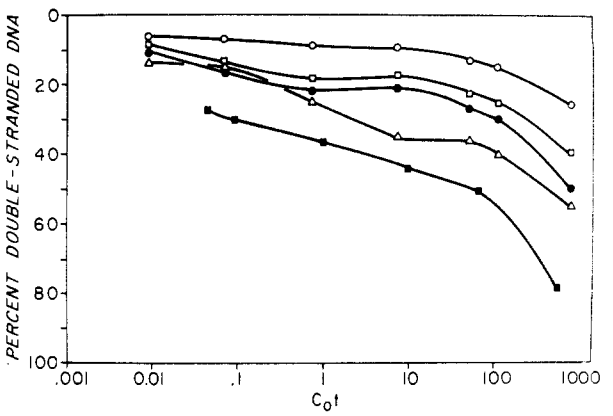

FIGURE 3: Molecular weight dependence of renaturation kinetics of mouse DNA assayed on hydroxylapatite. Labeled mouse L-cell DNA, diluted with unlabeled mouse embryo DNA to a specific activity of 300 cpm/μg, was degraded by depurination at 70° for various times as described in Methods. Samples were dilayzed, concentrated, and adjusted to 48% formamide, 5 × SSC at 2000 μg/ml. Aliquots of 50 μl sealed in capillary tubes were heated at 100° for 5 min and incubated at 37° for various times prior to assay on hydroxylapatite in the usual way. (○) 88-min depurinated (□) 44 min, (●) 22 min, (△) 5.5 min, and (■) sheared DNA. Molecular weights of the DNA may be estimated from the data presented in Table I.

RNA was isolated from *B. subtilis* strain WB 746 by essentially the same procedure, except that bacteria were broken by passage through a pressure cell at 12,000 psi.

Hybridization of RNA to DNA. In the mouse experiments, RNA concentrations were normally 20 mg/ml and labeled unique mouse DNA (60,000 cpm/μg) was present at a concentration of 1.5 μg/ml. Aliquots in 0.4 M phosphate buffer were sealed in capillary tubes and heated at 100° for 10 min prior to incubation at 70°. Bacterial DNA (0.2 μg/ml) and RNA (normally 1 mg/ml) in 48% formamide, 5 × SSC, were heated at 100° for 5 min immediately before incubating at 37° for hybridization kinetic studies. Aliquots were removed at various times, diluted into 5 ml of 0.12 M phosphate buffer and dialyzed against this same buffer. This step is essential in the case of all samples containing formamide. Hybridized DNA was determined as described below.

Separation of Single-Stranded from Hybridized DNA. Single-stranded DNA was separated from hybridized DNA on 2-ml hydroxylapatite columns. In general, 50-μl aliquots of reaction mixtures were diluted into 5 ml of 0.12 M phosphate buffer and loaded on columns at 60°. The flowthrough plus two 2-ml washes with 0.12 M phosphate was pooled and designated the single-stranded fraction. Material eluted from the column by three 2-ml washes with 0.5 M phosphate was designated the double-stranded fraction. Both fractions were precipitated with 10% trichloroacetic acid using 200 μg of bovine serum albumin carrier. Precipitates were collected on Whatman GF-C filters and counted in a Packard Tri-Carb liquid scintillation counter. The per cent of DNA hybridized was calculated by dividing the counts in the double-stranded fraction by the total number of counts. The counts in the double-stranded fraction were corrected for quenching due to the large amounts of RNA in them (usually 1 mg/filter). Background binding of labeled single-stranded unique DNA by hydroxylapatite remained relatively constant at about 2%, and background levels were subtracted from maximum per cent hybridization to arrive at actual per cent DNA hybridized.

Thermal Denaturation of Unique DNA–RNA Hybrids. The double-stranded fraction isolated following unique ^{3}H-labeled DNA incubation with mouse brain RNA was placed on a 3-ml hydroxylapatite column at 60° heated by a water bath. The temperature of the circulating thermoregulatory bath was raised by increments of 5° and after 5 min for equilibration, the column was eluted at each temperature with 5.0 ml of 0.12 M phosphate buffer.

Nucleic acids were precipitated in 10% trichloroacetic acid, collected on Whatman GF-C filters and counted in a Packard Tri-Carb liquid scintillation counter.

Hybridization of Pulse-Labeled L-Cell RNA with Total DNA. L-cell RNA pulse labeled for 16 min was incubated with 1000-fold excess of total mouse DNA in 48% formamide, 5 × SSC at 37°. Following dialysis against 4 × SSC, DNA–RNA hybrids were collected on Schleicher & Schuell B-6 membrane filters previously wetted in 4 × SSC, 0.1% sodium dodecyl sulfate, and washed with 100 ml of 4 × SSC. Filters were counted as before.

Results

Hybridization of B. subtilis RNA with DNA. The experimental design used in the studies of mouse RNA hybridization with unique sequences was based on that used previously by Gelderman *et al.* (1971) and Hough and Davidson (1971). In addition, a few model experiments were performed with bacterial nucleic acids both as a check on the procedure and for the purpose of determining the extent of transcription of the bacterial genome. *B. subtilis* was chosen because the per cent GC of its DNA is similar to that of mammalian DNA. [^{3}H]DNA was sheared to a single-stranded molecular weight of 4.2 × 10^5 and incubated (48% formamide, 5 × SSC at 37°) with unlabeled RNA at various concentrations. Samples were taken as a function of time and assayed on hydroxylapatite for the per cent duplexes. Using 1 mg/ml of RNA, the fraction of hybridized DNA reached a plateau at about 30% after some 12 hr (Figure 2). Lower concentrations of RNA

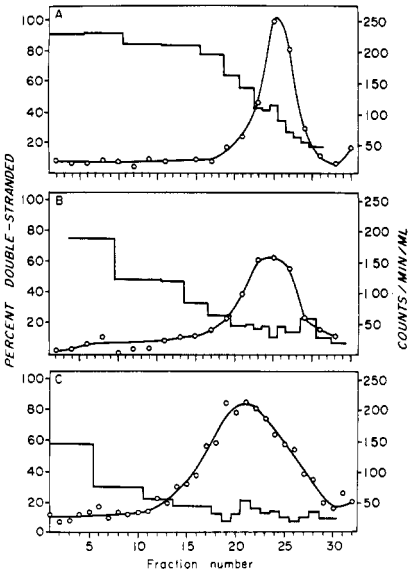

FIGURE 4: Retention of *B. subtilis* DNA–RNA hybrids on hydroxylapatite as a function of starting DNA molecular weight. *B. subtilis* DNA of three different molecular weights (sheared (A), 33-min depurinated (B) and 66-min depurinated (C)) were hybridized to saturation with RNA (0.2 mg/ml of DNA, 1.0 mg/ml of RNA, 48% formamide, 5 × SSC, 37°, 18 hr). Five milliliter of each reaction mixture was dialyzed against 0.1 × SSC, concentrated by evaporation to 4.0 ml and adjusted to a density of 1.72 by addition of 5.2 g of CsCl (Harshaw optical grade powder), and centrifuged in the SW-39 rotor for 60 hr at 20°. The indicated pooled or individual fractions were dialyzed against 0.12 M phosphate buffer and chromatographed on hydroxylapatite. The figure indicates the per cent of the material which elutes in the double-stranded peak from hydroxylapatite.

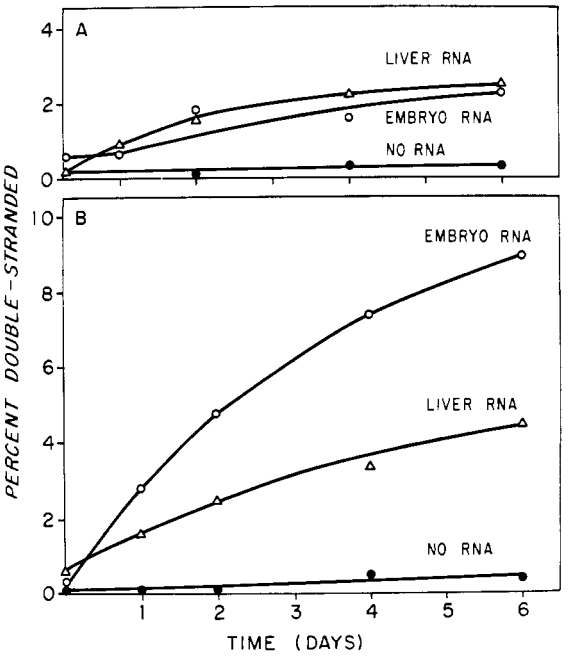

FIGURE 5: Hybridization of mouse RNA with labeled unique mouse DNA. (A) 1.78 μg/ml of ^{3}H-labeled unique mouse DNA was incubated with 20 mg/ml of total 18-day mouse embryo RNA (○), 20 mg/ml of adult liver RNA (△), and no RNA (●) at 50° in 38% formamide, 5 × SSC. (B) 1.78 μg/ml of ^{3}H-labeled unique mouse DNA was incubated with 20 mg/ml of total 18-day mouse embryo RNA (○), 20 mg/ml of adult mouse liver RNA (△), and no RNA (●) at 70° in 0.4 M phosphate buffer.

appeared to be insufficient to reach a saturation plateau. Higher concentrations of RNA yielded no increase in the per cent of DNA hybridized.

Thus, these results suggest that some 60% of the *B. subtilis* genome is transcribed and represented as RNA molecules in exponentially growing cells if one-strand transcription is assumed. Obviously, such estimates are minimal ones since a true plateau may not have been achieved. Nevertheless, this estimate is in reasonable agreement with the percent transcription in *E. coli* cells growing under similar conditions (Kennell, 1968).

Isolation of Unique Sequences of Mouse DNA. Initial attempts to isolate the unique sequences of the mouse genome by hydroxylapatite fractionation of partially renatured DNA gave surprisingly low yields of single-stranded unique fraction. Exploration of this highly reproducible observation brought to light the fact that mouse DNA renaturation kinetics as measured by hydroxylapatite are strikingly affected by the molecular weight of the DNA (Figure 3) (Table I). While the effect of molecular weight upon reaction rate is known (Wetmur and Davidson, 1968), large variation in the apparent percentage of redundant sequences is difficult to explain by kinetic arguments (Southern, 1971; Sutton and McCallum, 1971). The simplest interpretation of the data of Figure 3 is that DNA molecular weight must be reduced to about 1.7 × 10^5 in order to allow physical separation of unique from redundant sequences (Britten and Kohne, 1968). DNA of higher molecular weight contains unique and redundant sequences on the same fragments. Use of such higher molecular weight DNA for isolation of unique sequences would lead not only to low yield, but to specific loss from the unique category of those unique sequences which are contiguous with redundant sequences. Accordingly, the unique sequences used for the experiments to be described below were isolated from DNA of molecular weight 1.7 × 10^5 produced by 33-min depurination of 70°, pH 4.2, followed by alkali cleavage. Bacterial DNA of this molecular weight was found to behave similarly to sheared bacterial DNA in the hydroxylapatite assay for DNA–RNA hybrid, although lower molecular weight DNA (66-min depurinated, molecular weight 8 × 10^4) produced hybrid which exhibited markedly poorer binding in hydroxylapatite. That this is due largely to failure to bind, not failure to hybridize, is clear from Figure 4, which shows the ability of hybrids of various molecular weights to bind to hydroxylapatite. Hybridized DNA (sheared, 33-min depurinated and 66-min depurinated) was centrifuged to equilibrium in CsCl and pooled fractions from various density strata were dialyzed and tested for ability to bind to hydroxylapatite in the hybrid assay. Even the most dense fractions of hybridized 66-min depurinated DNA gave markedly poorer binding than 33-min depurinated and sheared DNA hybrids.

Hybridization of Mouse RNA with ^{3}H Unique Mouse DNA. Kinetics of hybridization of mouse liver and embryo RNA under two different reaction conditions are shown in Figure 5. Consistent with findings with the bacterial DNA–RNA hybrids (Figure 2), the use of formamide and high salt, which decrease the reaction temperature and increase rate in DNA–DNA reactions (McConaughy *et al.*, 1969) gave unexpectedly slow reaction rates (Figure 5a) compared to those attained in aqueous solution, 0.4 M PO_4 at 70°, (Figure 5b). This effect was most marked when RNA concentrations above 1 mg/ml were used. Aqueous reaction mixtures were therefore used for all subsequent experiments.

The effect of varying concentrations of brain RNA upon

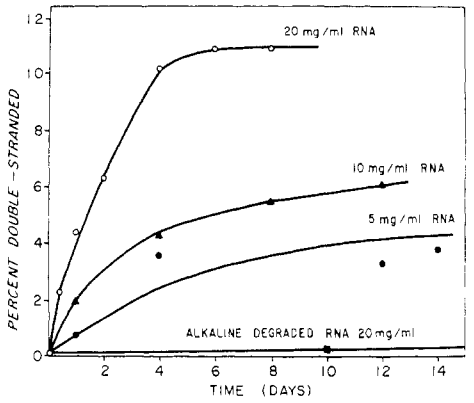

FIGURE 6: Hybridization of mouse brain RNA with mouse unique DNA. 1.78 μg/ml of unique ^{3}H-labeled mouse DNA was hybridized with 5 mg/ml (●), 10 mg/ml (▲), and 20 mg/ml (○) of mouse brain RNA. Also, 20 mg/ml of alkaline degraded mouse brain RNA (■) was hybridized with 1.78 μg/ml of unique ^{3}H-labeled mouse DNA.

the rate of hybridization of ^{3}H unique sequences is shown in Figure 6. The reactions showed the expected kinetics with the initial rate proportional to the RNA concentration. With 20 mg/ml of brain RNA, a plateau at 11% was achieved after about 1 week of incubation. At lower concentrations, the reactions continued for longer periods. That the reaction is exclusively one of DNA–RNA hybridization is shown by the fact that alkali treatment of the RNA eliminates all reaction. Thus, the reaction cannot be attributed to traces of DNA contaminating the RNA preparations.

Incubation at 70° under these conditions does degrade the RNA to some extent. RNA incubated for 9 days at 70° was run on a Sephadex G-200 column with various markers (Figure 7). The majority of the RNA eluted in the void volume, suggesting that a molecular weight greater than 50,000 was preserved (M. Farquhar, unpublished data). Davidson and Hough (1969) obtained similar G-200 profiles before and after incubation of their RNA preparations.

Since unlabeled RNA was used in all the experiments to be described, no direct estimate was possible of the amount of RNA in the DNA–RNA hybrid. This consideration is important since hydroxylapatite will recognize as double-stranded even molecules which contain a considerable degree

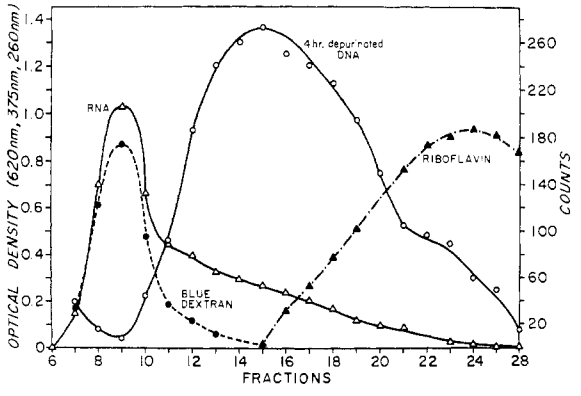

FIGURE 7: Sephadex chromatography of RNA following prolonged exposure to high temperature. Mouse brain RNA incubated at 20 mg/ml in 0.4 M phosphate buffer at 70° for 9 days, 4-hr depurinated ^{3}H-labeled mouse DNA, riboflavin, and Blue Dextran were chromatographed on a Sephadex G-200 column.

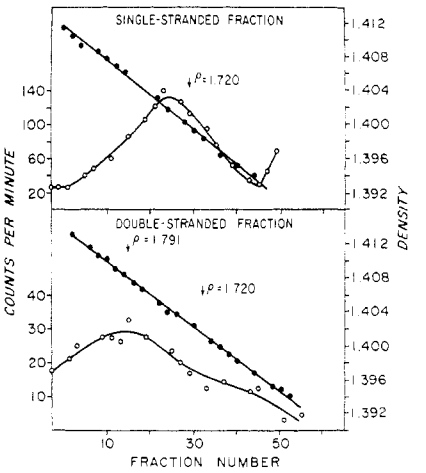

FIGURE 8: Buoyant density in CsCl of the single-stranded and double-stranded fractions from a hydroxylapatite column after hybridization of mouse brain RNA with mouse unique DNA. Mouse brain RNA (20 mg/ml) was incubated with labeled unique sequences of mouse DNA (1.5 μg/ml) in 0.4 M phosphate buffer at 70° for 8 days. A 50-μl aliquot was fractionated in the usual way on hydroxylapatite, and the single-stranded and double-stranded fractions were dialyzed against 0.015 M NaCl–0.0015 M citrate, concentrated by evaporation in an air stream to a volume of 4.0 ml, and adjusted to an appropriate density with solid CsCl. After centifugation for 3 days at 35,000 rpm in a Spinco SW-39 rotor, the gradients were fractionated and alternate fractions used for assay of radioactivity and measurement of refractive index.

of single-stranded ends. An approximate estimate of the stoichiometry of the DNA–RNA hybrid is possible, however, from a determination of the CsCl buoyant density. Aliquots of [^{3}H]DNA appearing in the single-stranded and the double-stranded fractions from hydroxylapatite after a DNA–RNA hybridization were centrifuged to equilibrium in CsCl. Although the peaks were broad due to the low molecular weight (Figure 8), a rough calculation of the stoichiometry is possible. The observed density of 1.791 at the peak compared to 1.720 for single-stranded DNA would suggest an average density increment of 0.071 g/cm^3. This density change may be compared to a difference between $\phi\chi$174 single-stranded DNA and 1:1 $\phi\chi$174 DNA–RNA hybrid of 0.095 g/cm^3 (Sinsheimer and Lawrence, 1964). This comparison would imply that the mouse DNA–RNA hybrids have a stoichiometry of approximately 1:0.75. More precise estimates are difficult to make since the density distribution is quite broad.

A sensitive criterion for the fidelity of base pairing in the DNA–RNA hybrids is provided by their thermal stabilities. Determination of the thermal stability of hybrids formed by unique mouse DNA and brain RNA after incubation to C_0t = 10,000 was made as described under Methods. The mean thermal stability appears to be about 81° (Figure 9). In very similar experiments with mouse brain DNA–RNA hybrids, Brown and Church (1971) obtained a value of 79° and Hahn and Laird (1971) a value of 82.5°. As these authors have argued, the hybrids dissociate at a temperature close to that expected for well-matched duplexes of this base composition and chain length. The T_m is considerably higher than that obtained for hybrids formed by redundant DNA at low C_0t values (Church and McCarthy, 1968).

Comparison of Reactions of RNA from Various Organs. As illustrated in Figure 6, some 11% of ^{3}H mouse DNA is capable of forming hybrid with brain RNA. In agreement with the findings of Brown and Church (1971) and Hahn and Laird

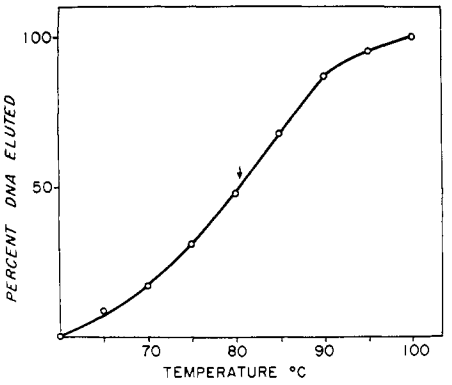

FIGURE 9: Thermal stability of mouse brain RNA–mouse unique DNA hybrids. The hybridized fraction isolated by hydroxylapatite following incubation of 1.78 μg/ml of unique, ^{3}H-labeled mouse DNA with 20 mg/ml of mouse brain RNA for 5 days was exposed to increasing temperature and the DNA eluted at each temperature with 0.12 M phosphate buffer was collected and counted.

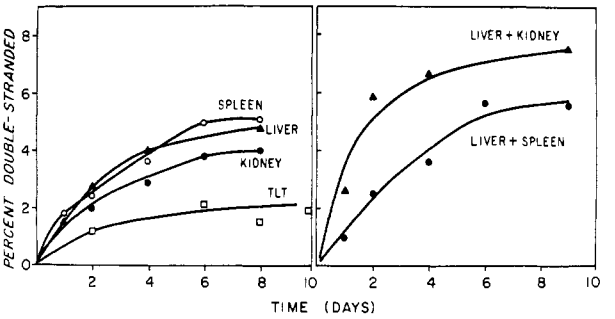

FIGURE 10: Hybridization of RNA from one or a mixture of two different mouse organs with mouse unique DNA. 1.78 μg/ml of unique ^{3}H-labeled mouse DNA was incubated with 20 mg/ml of (A, left) spleen (○), liver (▲), kidney (●), and TLT (□) RNA; and (B, right) 10 mg/ml of liver + 10 mg/ml of kidney (▲), and 10 mg/ml of liver + 10 mg/ml of spleen (●) RNA.

(1971), RNA of other mouse organs hybridized with considerably lower percentages of the DNA. For example, using the same concentrations and incubation times as those for brain RNA, kidney, liver, and spleen RNA all gave apparent saturation plateaus of 4–5% (Figure 10A).

It seems likely that these apparent saturation values reflect the diversity of cell types present in the organ in question, as much as the transcriptional diversity in a particular type of cell. This explanation is supported by the fact that still lower saturation plateaus were obtained with RNA isolated from a transplantable mouse hepatoma (TLT) (Taper *et al.*, 1966) (Figure 10A).

The fact that such similar saturation plateaus were obtained for spleen, liver, and kidney RNAs does not necessarily imply that these populations of RNA are very similar. The agreement might well be fortuitous, with different fractions of the genome being transcribed in each of these organs. This issue is resolvable in several ways, including tests of the additivity of the saturation plateaus and by means of recycling experiments.

A simple test of additivity was made by measuring the hybridization kinetics of a mixture of liver and kidney RNA or liver and spleen RNA. In both cases, an apparent saturation plateau was obtained which exceeded that obtained for RNA from either organ alone. This result implies that the populations of RNA molecules are partially, but not completely, overlapping (Figure 10B). This is to be expected if RNA molecules represent tissue specific gene products as well as enzymes or structural proteins common to many cell types.

A more sensitive approach to the question of overlapping populations of RNA molecules involves the isolation of the fraction of the DNA transcribed in a given organ and recycling with other RNA preparations. For example, the 11% of unique DNA complementary to brain RNA was physically separated by hydroxylapatite chromatography in the form of a DNA–RNA hybrid (Table II). After destroying the RNA moiety by alkaline hydrolysis, the single-stranded DNA was tested for its ability to react with various RNA preparations. As expected, the DNA showed a much greater affinity for brain RNA than did total DNA (Table II). However, the second reaction did not approach the expected 100%, presumably because the prolonged incubation caused the DNA to be degraded to a molecular weight below that required for discrimination on hydroxylapatite. The reaction with liver RNA was also appreciably higher than for total DNA, indicating that at least some of the DNA base sequences complementary to liver RNA are enriched by reaction with brain RNA. Again, the result is consistent with a partial overlap in the populations of RNA in the two organs in question.

Transcription in Brain as a Function of Age. In view of the great variety of RNA present in the mouse brain compared to other organs, it is of obvious interest to examine the RNA population present as a function of the age of the animal. In the case of brain RNA, the percentage of the genome transcribed increases dramatically with age both before and after birth (Figure 11). No significant change was observed when a parallel study was made with liver RNA isolated from animals of different ages. Whether this effect is directly related to changes in nervous function or simply to the later differentiation of the brain as compared to the liver remains to be seen.

Relative Transcription of Redundant and Unique DNA. The experiments described above deal only with the 70% of the mouse genome which is operationally unique under the conditions of the reaction. The remaining portion of the genome contains base sequences which are too closely interrelated to permit this kind of analysis. Thus, we cannot extend these estimates of relative transcription to the total genome unless we assume that the 70% unique DNA is representative of all nonsatellite base sequences. Although this assumption cannot be supported by conclusive evidence, it is possible to show

TABLE II: Reincubation of DNA Which Does (0.5 M Fraction) and Does Not (0.12 M Fraction) Form Hybrids with Brain RNA.

Expt	Source of RNA for Reincubn	Percentage of DNA-Forming Hybrid: 0.12 M Fraction	Percentage of DNA-Forming Hybrid: 0.5 M Fraction
1	Brain	2.7	30.9
	Liver	3.2	15.3
	None	2.5	3.0
2	Brain	1.8	33.2
	Liver	2.3	12.9
	None	2.3	5.6

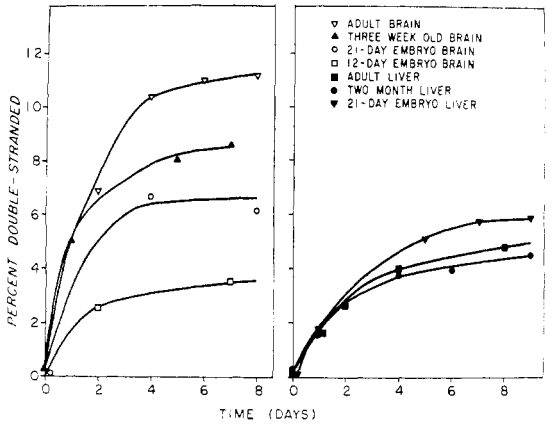

FIGURE 11: Hybridization of mouse brain and liver RNA as a function of age. 1.78 μg/ml of unique ^{3}H-labeled mouse DNA was incubated with 20 mg/ml of (A, left) adult mouse brain RNA (▽), 3-week-old mouse brain RNA (▲), 21-day mouse embryo brain RNA (○), 12-day mouse embryo brain RNA (□); (B, right) and with adult mouse liver RNA (■), 2-month-old mouse liver RNA (●), and 21-day mouse embryo liver RNA (▼).

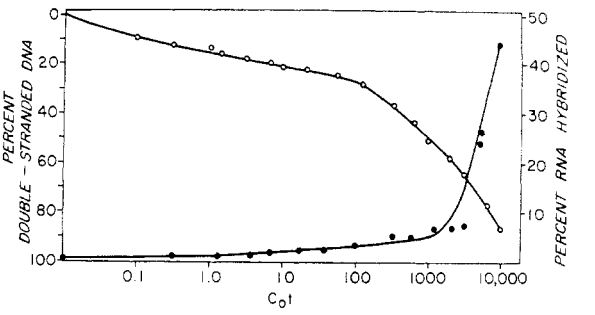

FIGURE 12: Kinetics of a DNA driven reaction between pulse-labeled RNA and unlabeled DNA. 2 mg/ml of sheared mouse DNA was incubated in 48% formamide, 5 × SSC at 37° with 1.9 μg/ml of 16-min pulse-labeled L-cell RNA with a specific activity of 8000 cpm/μg. Per cent hybrid formed was measured by collecting hybrids on membrane filters as described in Materials and Methods.

that redundant and unique sequences are transcribed to roughly the same extent.

Figure 12 illustrates an experiment in which a low concentration of ^{3}H mouse L-cell RNA was incubated with a great excess of unlabeled mouse DNA. In this reaction, the kinetics of hybridization are governed by the DNA concentration. The incubation was carried out in formamide at 37° since the kinetic problems encountered with high concentrations of RNA (Figure 5) are not observed when high concentrations of DNA are employed (McConaughy *et al.*, 1969). It can be seen that most of the hybridization occurs at values of C_0t characteristic of the renaturation of unique DNA. A similar finding was reported earlier for L-cell nuclear and cytoplasmic RNA (McCarthy *et al.*, 1969). In fact, in the present experiment it appears that the proportion of the RNA hybridizing in this range of C_0t is greater than the fraction of DNA represented by unique sequences, as though unique sequences were preferentially transcribed. However, it should be noted that since redundant hybrids have a lower T_m than those with unique DNA, the conditions of reaction are in fact more stringent for redundant DNA–RNA hybrids. This effect would tend to bias the results toward an apparently higher proportion of reaction with unique DNA. Despite these complications, we conclude that neither unique nor redundant sequences are preferentially transcribed. Kedes and Birnstiel (1971) have recently reported that most of the RNA present in sea urchin embryos is representative of unique DNA sequences, which also constitute the majority of that genome.

Discussion

In evaluating the experimental approach described above, the primary concern is to determine whether the observed reaction plateau values truly reflect varying degrees of transcription in the tissues studied. In the case of the brain RNA reaction at 20 mg/ml, we have shown that the reaction is completed, and that adding fresh RNA of the same type produces no further hybridization (Table II). The brain RNA reaction is essentially complete in 4 days at 20 mg/ml. Simple kinetic considerations would lead one to expect that 10 mg/ml of brain RNA would attain a similar plateau value in about 8 days. The fact that this is not seen (Figure 6) suggests that at some time around or after 4 days of incubation, the RNA or the DNA is sufficiently degraded to affect its rate of reaction. Thus, any reaction not fast enough to go to completion in about 4 days in 0.4 M PO_4 at 70° can yield an artifactual plateau value. The primary factor affecting reaction rate is, of course, the concentration of the hybridizing RNA in question. Since the concentration of total RNA was 20 mg/ml in most of the experiments reported, variations in the ratio of an mRNA or other readily hybridizable species to rRNA from one tissue to another will produce variations in the rate of hybridization and influence the apparent plateau value. Even if the ratio of total readily hybridizable RNA to rRNA is similar in different cells, the distribution among different messengers or other RNA species could be quite variable. Again, limits will be set by the solubility of RNA under the reaction conditions so that hybridization of low-frequency RNA species may not even approach completion. Unfortunately, it is very difficult to rule out this type of interpretation, and the validity of plateau estimates is defensible only by recycling experiments.

These cautionary considerations lead to the conclusion that these estimates of the transcriptional activity occurring in various mammalian tissues are far from precise. However, the estimates obtained do present minimal values and do warrant some discussion. In the first place, they are surprisingly large. The potential information present in the large genomes of mammals is so extensive as to provide for enormous numbers of different proteins, if sizeable fractions of it are transcribed and translated. However, there is no justification for equating the diverse set of RNA molecules with messengers; it would seem most likely that the majority of the sequences are representative of heterogeneous nuclear RNA whose function is presently obscure. It is also surprising that the proportion of the genome transcribed in the mouse brain, for example, about 11%, is so much higher than that reported for the mature Xenopus oocyte, 0.6% (Hough and Davidson, 1971). In the latter case, the RNA includes a variety of messengers stored and utilized later in embryonic development. Again, however, meaningful comparison is difficult since we have no way of estimating mRNA itself.

The fact that saturation plateaus are so much higher for brain than for other tissues is perhaps not so surprising. The high activity of the brain in both RNA and protein synthesis is well known, as is the complex organization of different cell types in the brain. We are as yet unable to specify whether glial cells, neuronal cells, or both contribute this high tran-

scriptional diversity. However, parallel experiments with human brain nucleic acids indicate that highly diverse populations of RNA molecules are not restricted to a particular part of the brain. On the contrary, several different regions of the human brain show transcriptional activities even more diversified than those presently reported for mouse brain (L. Grouse and B. J. McCarthy, in preparation, 1971).

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Enzymatic and Physical Studies on $(dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$ and $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$*

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ABSTRACT: Studies on the synthesis and characterization of $(dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$ are reported. The DNA is rereplicated much more efficiently at high pH values (9.3) than at lower pH values (7.4); in contrast, the sequence isomeric DNA, $(dI)_n\cdot(dC)_n$, is rereplicated more rapidly at the lower pH value. $(dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$ possesses unusual X-ray diffraction and circular dichroism properties; hence, additional physical and enzymatic studies with this polymer are reported. The DNA is an efficient template or substrate for a variety of DNA-metabolizing enzymes including polymerases, nucleases, kinase, and ligase. DNA ligase from T4-infected *E. coli* readily forms circular $(dI\text{-}dC)_n$ in high yield; this reaction may be used as a sensitive assay for DNA ligase. Viscometric studies on linear $(dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$ show that it readily undergoes a salt-facilitated strand rearrangement prior to the helix to coil transition, as shown for $(dA\text{-}dT)_n\cdot(dA\text{-}dT)_n$. Pancreatic DNase specifically cleaves $(dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$ at the IpC linkage to give oligomers which are terminated at the 5′ end with pC residues. The effect of chain length on the melting and circular dichroism properties is reported. Studies on the synthesis of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ are reported. The synthetic reaction demonstrates linear kinetics with either $(dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$ or $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ as templates and with either the *Micrococcus luteus* or the *Escherichia coli* DNA polymerase. The rate of the synthetic reaction is a function of the template nucleotide concentration and not the number of free 3′-OH ends. $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ is the most thermostable DNA found to date. Titration studies indicate that, at neutral pH, the base pairs are the normal Watson–Crick type. This DNA is insusceptible to degradation by exonuclease I.

A systematic study on the properties of DNA polymers is being performed in an effort to understand the effect of primary nucleotide sequence on the properties and structure of DNA (for a summary of these studies, see Wells *et al.*, 1970). We postulated that certain types of nucleotide sequences may serve as recognition sites for the regulation of genetic information. Fourteen different double-stranded DNAs contain-

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