Functional Significance of Rat Liver Nonhistone Protein–DNA Interactions: RNA Hybridization of Protein-Bound DNA†

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ABSTRACT: The complexity of polysomal messenger RNA (mRNA) has been measured by saturation hybridization analysis of purified whole single-copy DNA and single-copy sequences enriched from liver nonhistone-bound sequences of the rat genome. Nine and two-tenths percent (2.5×10^8 nucleotide pairs) of the whole single-copy component of the rat genome is complementary to liver mRNA, while 4.3% (1.2×10^8 nucleotide pairs) is complementary with kidney mRNA. Liver nonhistone proteins have been fractionated by selective solubilization (0.35–1.0 M sodium chloride) from purified liver nuclei and have been shown to interact with sheared rat DNA in vitro. It has also been demonstrated in these studies that nonhistone-bound DNA can be fractionated into repetitive and single-copy components. The bound single-copy component (2.9×10^8 nucleotide pairs) is 43% complementary with liver mRNA and 31% complementary with kidney mRNA. The rate of hybridization of the nonhistone-bound single-copy DNA component with liver mRNA is threefold faster than that with kidney mRNA, suggesting that the nonhistone–DNA interactions enrich transcribed sequences that are transcribed with greater efficiency in rat liver than kidney tissue.

Rat liver nonhistone proteins (NHP) can interact with repetitive sequences in the rat genome (Sevall et al., 1975) which can be assayed by following the reassociation kinetics (Jagodzinski et al., 1978). With increased DNA fragment length, the nonhistone protein-bound DNA is enriched with a subset of single-copy DNA sequences which neighbor the repetitive protein-binding site. This subset of sequences is enriched in transcribed single-copy sequences in rat liver mRNA (Jagodzinski et al., 1979).

mRNA transcripts consist of three frequency classes as determined from the kinetics of hybridization of poly(A)-containing mRNA and its complementary DNA (cDNA) (Getz et al., 1975; Levy & McCarthy, 1976; Axel et al., 1976; Monahan et al., 1976a,b). Saturation hybridization of single-copy DNA with excess poly(A)-containing mRNA hybridizes with a rate determined by the highest complexity component of both rat liver (Savage et al., 1978) and sea urchin (Galau et al., 1974) RNA. The high complexity, low abundant component of mRNAs represents ~50% of the mass of the mRNA population. However, those sequences that represent a small degree of complexity but a large amount of the mass of mRNA have been shown to translate into the major contractile proteins in differentiated myoblasts (Paterson & Bishop, 1977). This observation of specialized cellular function due to an accumulation of a large number of copies of a small number of mRNA sequences is consistent with comparisons of chicken oviduct and liver mRNA (Axel et al., 1976) and a variety of tissues in the mouse (Hastie & Bishop, 1976). The efficiency of transcription of an active gene is due to factors other than just the structural conformation of the transcribed gene (Miller et al., 1978).

We demonstrate in the present experiments that the nonhistone protein-bound DNA is enriched in single-copy sequences which are hybridized by excess rat liver mRNA at a greater rate than rat kidney mRNA. This suggests that a group of rat mRNA species which are present in greater frequency in rat liver than in kidney are the major driving species in the liver hybridization. These experiments suggest that the nonhistone DNA-binding proteins may be responsible for the greater efficiency in transcription of these liver genes.

Experimental Procedures

Isolation of Chromosomal Components. The purification of rat liver NHP has been described previously (Jagodzinski et al., 1979). Nonhistone proteins eluted between 0.35 and 1.0 M sodium chloride from purified nuclei represent 3.7% of the nuclear protein, and when mixed at ratios of 3:1 with 3400 nucleotide pair DNA (1 μg/mL), 0.24 M ionic strength (pH 8.0), 5.0% of the DNA is retained as protein-bound DNA.

Rat DNA was purified from crude chromatin by a modified Sevag procedure (Wu et al., 1972). The DNA was mechanically sheared to 3400 nucleotide pairs in a Virtis 60 homogenizer at 5000 rpm for 30 min in 0.2 M sodium acetate at 0 °C. DNA was sized over 5–20% alkaline sucrose gradients, and weight-average single-stranded lengths were determined according to the relationship derived by Studier (1965).

The procedure which we used for the isolation and characterization of undegraded polysomes and poly(A)-containing mRNA has been described in detail (Sala-Trepat et al., 1978). The tissue was homogenized with nuclease inhibitors: ammonium chloride, heparin, 2-mercaptoethanol, and yeast tRNA (1.5 mg/mL).

DNA Labeling. A microscale procedure to synthesize and purify rat DNA to high specific activity has been modified from the technique of Schachat & Hogness (1973) (Mackey et al., 1977). [^{3}H]DNA of specific activity 1.5×10^6 cpm/μg was prepared by nick translation by using 0.1 nmol of commercial [^{3}H]thymidine 5′-triphosphate (50 Ci/mmol, ICN), 0.4 nmol of deoxyguanosine 5′-triphosphate and deoxyadenosine 5′-triphosphate (P. L. Biochemicals), 66.7 mM potassium phosphate (pH 7.5), 6.7 mM magnesium chloride, 1 μg of unlabeled DNA, and 10 units of DNA polymerase I (Worthington Biochemicals or P. L. Biochemicals). The final volume was 25 μL. After 6 h of incubation at 14 °C, 0.1 mL of 0.1 M EDTA was added to the mix which was extracted and the double-stranded DNA was retained on a hydroxylapatite column. The labeled DNA was eluted in 0.48 M sodium phosphate, pH 6.8. Carrier DNA was added to the labeled DNA used in reassociation studies, and the mixture

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Table I: Analysis of mRNA Content in Polysomal RNA

tissue	preparations	polysomal RNA (mg)	poly(A) mRNA[c] [oligo(dT)–cellulose] (μg)	% mRNA[d]	% mRNA[e]
liver[a]	5	132 ± 13	2025 ± 185	2.1 ± 0.2	2.0 ± 0.3
kidney[b]	4	66 ± 6	990 ± 110	2.2 ± 0.2	1.9 ± 0.2

[a] The average of 6 rat livers/preparation. [b] The average preparation of 15 pairs of kidney. [c] By the method of Bantle et al. (1976). [d] % poly(A) mRNA was corrected for nonpoly(A) mRNA content, which is ~30% of the total mass of RNA. (Sala-Trepat, 1978). [e] Measured with [^{3}H]oligo(U) [Miles Labs (300 μCi/μmol)] by the method of Bishop et al. (1974b), corrected for ~30% nonpoly(A) mRNA.

was phenol-extracted, treated with chelex, and prepared for reassociation.

The labeled DNA has been characterized by its reassociation properties. The renaturation rate, complexity, and component composition of labeled DNA agree with those of unlabeled DNA. λ phage DNA restriction fragments were not altered by the nick translation of λ phage, indicating that the procedure does not alter the DNA.

cDNA from poly(A)-containing mRNA was synthesized with AMV reverse transcriptase (gift of J. Beard) as described by Monahan et al. (1976a). Long cDNA probes were selected by Sephadex G-200 chromatography of the reaction mixture diluted in 0.1 M trisodium phosphate, pH 11.0.

Nucleic Acid Reassociation or Hybridization. Samples were denatured at 100 °C for 10 min and annealed in sodium phosphate buffer (PB) at 62 °C for 0.12 M PB, 72 °C for 0.48 M PB, or 54 °C for 0.05 M PB. In most experiments both labeled and unlabeled DNAs were present in the reassociation mixtures. In such mixtures, the unlabeled DNA was present at greater than 10^4 times the concentration of labeled "tracer" DNA (Britten & Smith, 1970).

The reassociating nucleic acid was incubated to different C_0t or R_0t values [time (second) multiplied by DNA or RNA concentration (moles of nucleotides/liter)] calculated for the driver nucleic acid. After incubation, the samples were frozen in dry ice–ethanol for further analysis or immediately analyzed for double-stranded content as follows. The samples were diluted in 2–3 mL of 0.12 M PB and passed over a hydroxylapatite column (Bio-Rad, DNA grade) at 60 °C. Single-stranded nucleic acid was eluted in 10 mL of 0.12 M PB. The duplexed nucleic acid was eluted with 10 mL of 0.48 M PB. Fractions of 5 mL were collected, 100 μg of unlabeled carrier RNA (Sigma Chemical Co.) was added to the fraction, and the nucleic acid precipitated in ice-cold 10% trichloroacetic acid. The precipitated nucleic acid was collected on 24-mm diameter B6 Schleicher and Schuell membrane filters (0.45-μm pore size), washed with 66% ethanol, dried in a vacuum oven, and counted in a xylene base scintillation cocktail. The percent of single-strand DNA was determined from total counts recovered. The data for a single reassociation study were analyzed on a computer program capable of fitting several kinetic components with second-order or first-order kinetics (Pearson et al., 1977).

RNA-driven hybridizations with excess RNA and trace amounts of [^{3}H]DNA were denatured by heat and incubated in 0.41 M PB and 0.1% $NaDodSO_4$ at 62 °C to the calculated RNA R_0t. The hybridization mixtures were diluted in 0.12 M PB and chromatographed on hydroxylapatite as in the DNA–DNA reassociation experiments. To assay for DNA–DNA self-reassociation, we chromatographed a sample over Sephadex G-100 (0.3 × 20 cm) in 0.05 M PB. The nucleic acid peak was digested with 10 μg/mL RNase A overnight at 37 °C and assayed for DNA–DNA duplexes. The amount measured never exceeded the calculated self-reassociation of the tracer DNA.

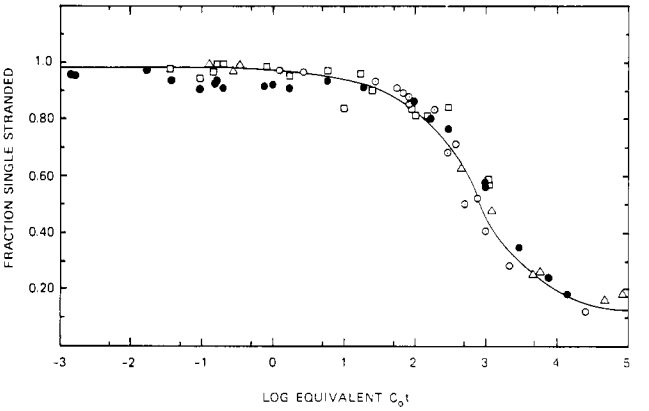

FIGURE 1: Reassociation profile of cDNA with total rat liver DNA. cDNA to poly(A) mRNA from rat liver was prepared as in the text. cDNA was reassociated with a 10^4-fold excess of total rat DNA. Duplexes were assayed by S_1 nuclease digestion (DIG = 80, coefficient of digestion)(Britten et al., 1977). All data were normalized to 0.12 M PB at 62 °C. The line drawn through the data represents the best least-squares fit.

General Methods. Protein concentrations were determined by the method of Lowry et al. (1951) or by the method of Bradford (1976) with bovine serum albumin (BSA) as the standard. DNA concentrations were determined spectrophotometrically by using an extinction coefficient of 6600 (L M^{-1} cm^{-1}) for the native DNA or 8850 (L M^{-1} cm^{-1}) for single-stranded nucleic acids.

Results

Characterization of the RNA Driver. To compare the sequence complexity of transcribed, single-copy DNA tracers, it was necessary to well characterize the RNA employed in the hybridizations. Undegraded rat liver and kidney polysomes were obtained as described in the Experimental Procedures (Sala-Trepat et al., 1978). The average size for poly(A)-containing mRNA from purified polysomal RNA and from poly(A)-containing mRNA isolated by oligo(dT)–cellulose chromatography (Bantle et al., 1976) was determined to be 1500 nucleotides by dimethyl sulfoxide denaturing sucrose gradients. The poly(A)-containing mRNA content in polysomal RNA was measured by two independent assays: (1) oligo(dT)–cellulose and (2) [^{3}H]poly(U) titration. Table I indicates that both liver and kidney polysomal RNA contain 2.1% mRNA, which was used to correct the polysomal RNA for mRNA content.

Poly(A)-containing mRNAs were characterized for sequence complexity by synthesizing cDNA probes with AMV reverse transcriptase (Monahan et al., 1976a). The cDNA was 350–400 nucleotides in length as measured on alkaline sucrose gradients with known DNA markers. A 2×10^5 excess of sheared (350 nucleotide pair) whole rat DNA (Figure 1) confirms that this cDNA consists of sequences transcribed from both repetitive and single-copy components of the rat genome. Fifteen percent of this cDNA reassociates with a

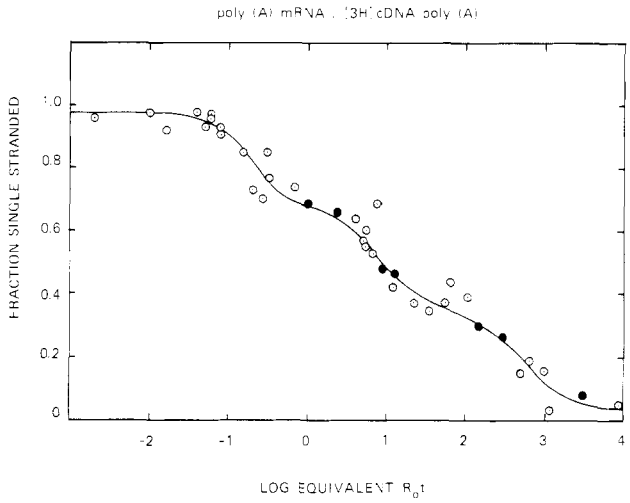

FIGURE 2: Hybridization between rat liver cDNA and its poly-(A)-containing mRNA template. Each point contained 5000 cpm of cDNA and a 100:1 mass ratio of poly(A)-containing mRNA/cDNA. The reactions were analyzed by using S_1 nuclease (Britten et al., 1977), and the data were analyzed by computer (Pearson et al., 1977) (Table II).

Table II: Frequency Classes in Rat Poly(A)-Containing mRNA

liver component[a]	% RNA	$R_0t_{1/2}$ (obsd) (M s)	k (M^{-1} s^{-1})	no. of sequences
1	30.0	0.018	38.4	6
2	32.0	7.8	0.092	2500
3	32.0	500	1.4×10^{-3}	1.7×10^5
kidney component[b]	% RNA	$R_0t_{1/2}$ (M s)	k (M^{-1} s^{-1})	no. of sequences
1	10.0	0.030	11.5	4
2	45.0	11.5	0.03	7700
3	45.0	22.0	0.0016	1.4×10^5

[a] In these studies, the cDNA was ~350 nucleotides in length with a mRNA length of 1500 nucleotides. [b] The length of the kidney poly(A)-containing mRNA was 1500 nucleotides.

$C_0t_{1/2}$ = 60 M s, and 85% reassociates with a $C_0t_{1/2}$ = 1100 M s. The majority of the cDNA is transcribed from the single-copy rat DNA component. This agrees with poly-(A)-containing mRNA isolated for rat liver (Campo & Bishop, 1974; Ryffel & McCarthy, 1975; Savage et al., 1978).

To determine the distribution of the complexity of the mRNA, we followed the kinetic analysis of reassociation of rat liver poly(A)-containing mRNA with its cDNA (shown in Figure 2). Analysis of the first-order hybridization kinetics shows three classes of RNA species. The values of the $R_0t_{1/2}$ and the fraction in each class are shown in Table II for the total amount of cDNA hybridizable (96%). The approximate number of sequences in each class was calculated relative to a known standard used in this laboratory, globin mRNA (k = 577 M^{-1} s^{-1}, 600 nucleotides). The average size of the poly(A)-containing mRNA was 1500 nucleotides. The values obtained for the length of kidney poly(A)-containing mRNA and the complexity distribution are tabulated in Table II.

mRNA-driven hybridizations of trace amounts of ^{3}H-labeled single-copy DNA reassociate with a rate which is determined by the high complexity class of transcripts (Galau et al., 1974). Figure 3 shows liver and kidney mRNA-driven hybridization of trace amounts of single-copy rat DNA.

The saturation values for liver and kidney mRNA hybridizations are reported in Table III as 4.6 and 2.2%, respectively, with DNA complexities of 2.5×10^8 and 1.2×10^8

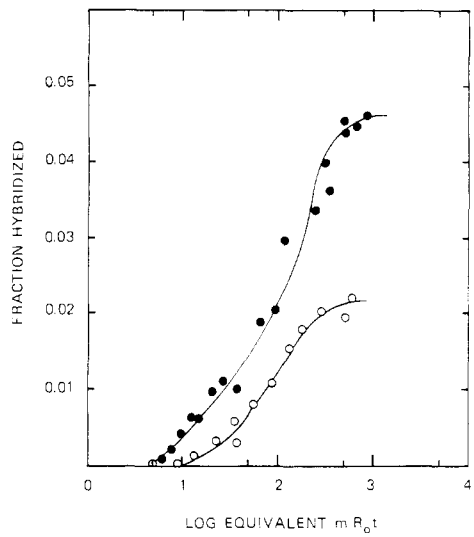

FIGURE 3: Hybridization of single-copy DNA with polysomal mRNA. Single-copy DNA was isolated from the total rat genome by two successive reassociations to C_0t = 200.0 M s. The single-copy DNA was reassociated to C_0t = 20 000 M s, labeled in vitro by DNA polymerase I, and hybridized with a 10^4-fold excess of polysomal mRNA. All data were normalized to 0.12 M PB at 62 °C. (●) Liver polysomal mRNA; (○) kidney polysomal mRNA. Polysomal RNA contains 2.1% mRNA (Table I), and R_0t values were calculated based on mRNA content.

Table III: mRNA-Driven Hybridizations[a]

mRNA	% hybridized	$R_0t_{1/2}$ (obsd) (M s)	% RNA driving hybridization	goodness of fit
Whole Single-Copy Tracer (Complexity = 2.8×10^9 nuc)				
liver	4.3	104.0	50	0.0024
kidney	2.3	104.0	66.9	0.0010
Bound Single-Copy Tracer (2.9×10^8 nuc)				
liver	18.4	35	28.7	0.0150
kidney	17.0	104.2	100	0.0105

[a] Parameters were determined by a general computer program of a nonlinear least-squares method (Pearson et al., 1977). The parameters are reported as an "unconstrained fit" where the lowest goodness of fit parameter was obtained.

nucleotide pairs, respectively. From the measured complexities, the expected rate of hybridization can be calculated and the ratio of the expected rate to the observed rate (Table III and Figure 3) determines the amount of mRNA driving the hybridization (Galau et al., 1974; Davidson, 1976). Table III indicates that 50% of the mRNA in the liver preparation drives the reassociation. Thirty-two percent of liver mRNA is found in the large sequence class (highest complexity class) of the poly(A)-containing mRNA, which is near the amount of RNA that is observed driving the RNA excess hybridization. The kidney-driven hybridization has 67% of the RNA driving the hybridization with 45% found in the high complexity class of kidney mRNA. These results indicate that the driven hybridizations are dependent on the class of RNA that contains the greatest amount of complexity as the predominant source of hybridization.

Characterization of the Nonhistone Protein-Bound DNA Tracer. Chromatin from purified nuclei can be selectively extracted with increasing ionic strength (Comings, 1978; Bloom & Anderson, 1978), releasing subclasses of nonhistone proteins. A 0.35–1.0 M sodium chloride extraction of purified nuclei releases 3.7% of the chromosomal proteins. These moderate salt-extracted proteins are passed over a Bio Rex-70 cation-exchange column in 0.4 M sodium chloride and 0.01

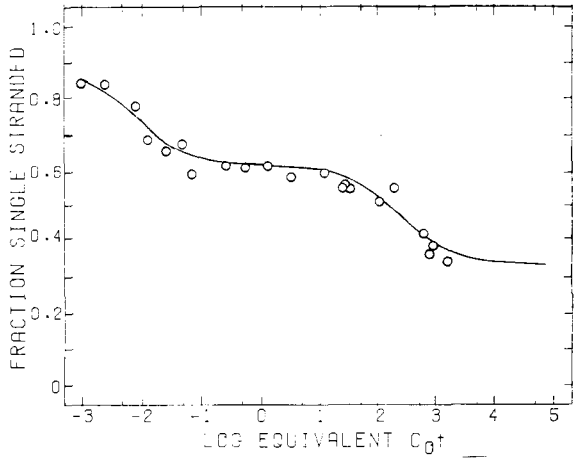

FIGURE 4: Reassociation profile of 3400 base pair protein-bound DNA sheared to 350 nucleotides. Protein-bound DNA (3400 base pairs) was isolated by nitrocellulose membrane filtration at 3 μg of protein/(μg of DNA mL^{-1}). The protein-bound DNA (BDNA) was sheared to 350 nucleotides by two passes through an Aminco French pressure cell at 10000 psi. Purified BDNA was self-reassociated in 0.05, 0.12, and 0.48 M phosphate buffers (PB) at 56, 62, and 72 °C, respectively. All data were normalized to 0.12 M PB at 62 °C. DNA duplexes were assayed by hydroxylapatite chromatography (Britten et al., 1974). The line through all data describes the best least-squares fit.

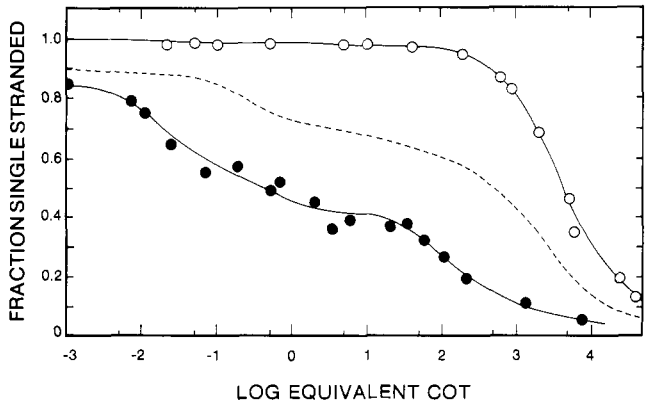

FIGURE 5: Reassociation profile of trace amounts of the fast (●) and slow (○) components of the bound DNA in the presence of an excess of unlabeled total rat DNA (---). ^{3}H-Labeled bound DNA sheared to 350 nucleotides was reassociated to C_0t = 1000 M s, and the double-stranded DNA was isolated and again renatured to C_0t = 1.0 M s. The double-stranded DNA and the single-stranded DNA at C_0t = 1.0 M s were isolated by hydroxylapatite chromatography and both components reassociated with a 5000-fold excess of total rat DNA. All data were normalized to 0.12 M PB at 62 °C. DNA duplexes were assayed by hydroxylapatite chromatography (Britten et al., 1977). The line through the data describes the best least-squares fit.

M Tris-HCl (pH 8.0) to remove any solubilized histones (Levy et al., 1972; van den Broek et al., 1973). At protein/DNA mass ratios of 3:1 and DNA concentrations of 1 μg/mL, 5% of the total amount of DNA can be retained on a nitrocellulose membrane filter. The DNA can be isolated, repurified, and sheared to 350 nucleotide pairs in a French pressure cell at 20000 psi (66% glycerol, 0.1 M sodium acetate). The self-reassociation of these fragments indicates that two major reassociating components are present (Jagodzinski et al., 1979) (Figure 4). The fast component reassociated with a $C_0t_{1/2}$ = 0.01 M s and the slow component with a $C_0t_{1/2}$ = 256 M s. The slow component was isolated by reassociating ^{3}H-labeled, sheared, bound DNA to a C_0t = 1000.0 M s, isolating the double-stranded DNA, and reassociating this DNA again to a C_0t = 1.0 M. The single-stranded DNA was then collected and, as shown in Figure 5, reassociated to 95% with the single-copy component in an excess whole genome driven reassociation. The isolated nonhistone-bound single-copy DNA has a complexity of 2.8 × 10^8 nucleotides as determined from its self-reassociation rate.

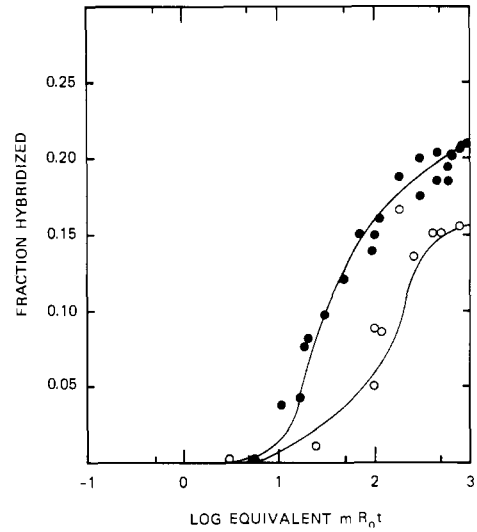

FIGURE 6: Hybridization of single-copy bound DNA with liver and kidney polysomal mRNA. Single-copy bound DNA (Figure 2) was hybridized with a 5000-fold excess of polysomal mRNA. All data were normalized to 0.12 M PB at 62 °C. RNA–DNA duplexes were assayed by hydroxylapatite chromatography. Lines are drawn to a first-order fit. mRNA R_0t values were calculated from polysomal RNA content. (●) Liver polysomal mRNA; (○) kidney mRNA.

Polysomal RNA Driven Reassociation of the NHP-Bound DNA. Figure 6 shows the hybridization of ^{3}H-labeled single-copy nonhistone-bound component with excess mRNA. The liver and kidney mRNA hybridizations have saturated nonhistone-bound single-copy DNA at 21.5 and 15.6%, respectively. The DNA complexity of the bound DNA is 9.0 × 10^7 nucleotides for kidney RNA and 1.2 × 10^8 nucleotides for liver RNA, which represents 64 and 46%, respectively, of the complexity observed with the whole single-copy tracer. The rate of hybridization, which is related to the $R_0t_{1/2}$, is 110 M s for the kidney RNA. This is observed when the whole single-copy component is reassociated by the high complexity class of kidney mRNAs. Liver RNA has a $R_0t_{1/2}$ of 35 M s, which is threefold faster than that expected for the high complexity class of mRNAs. The mass of liver mRNA driving the hybridization is 28.7%, which approximates the mass amount of individual classes in liver mRNA. The mass amount of kidney mRNA driving the reaction is near 100%. Since kidney RNA has two mRNA classes that make up 90% of the entire mass, the observed rate indicates that both classes are driving the hybridization. Thus, liver nonhistone-bound single-copy DNA contains sequences that are complementary to a subset of liver mRNA complexity, but which are hybridized by the more complex classes of kidney mRNA.

Discussion

RNA-driven hybridizations can measure the complexity of an RNA population and can compare the sequences present in various RNA populations. The limiting criterion in this technique is the saturation plateau of hybridization (Galau et al., 1976). In the present study, various criteria indicate that saturation has been achieved. First, the tracer DNA, either whole single-copy DNA or single-copy NHP-bound DNA, has been shown to react with total DNA-driven reassociations to greater than 90%. Second, the RNA pop-

ulations were shown to have a similar level of poly(A)-containing nucleotides, which represents 70% of the functional mRNA [poly(A)-containing + poly(A)-minus mRNA] (Sala-Trepat et al., 1978). Third, termination was determined by hybridizing to R_0t values that were 100 times the $R_0t_{1/2}$ values with no change in the amount of DNA hybridized. Also, the same saturation values were obtained with three different populations of the reacting components at different RNA/DNA mass ratios (Davidson et al., 1973).

The mRNA preparation was characterized by measuring the diversity and complexity of the poly(A)-containing mRNA by hybridization with its cDNA (Bishop et al., 1974a). cDNAs synthesized from the poly(A)-containing message hybridized to 85% with excess rat DNA (Campo & Bishop, 1974). With an excess poly(A)-containing mRNA as assayed by S_1 nuclease, 96% of the cDNA was hybridized. From these data, three classes of mRNAs were characterized for rat liver and kidney mRNAs which reflect the apparent frequency of the mRNA transcript. The standard used in the quantitation of these data was globin mRNA and its cDNA which reassociated with $k = 577\ M^{-1}\ s^{-1}$ and a length of 600 nucleotides. The messenger classes and complexities agree with previously reported values (Hastie & Bishop, 1976; Ryffel & McCarthy, 1975; Young et al., 1976; Axel et al., 1976; Savage et al., 1978). The slightly higher numbers of gene transcripts may be due to the use of globin mRNA as a standard, which is one-half the measured size of the liver mRNA (Hereford & Rosbash, 1977).

The complexity measured by single-copy DNA saturation hybridization by the mRNA of liver or kidney gives values that agree with those of the poly(A)-containing mRNA–cDNA hybridizations. The mass of RNA driving the reaction in the saturation hybridization assay is 50% for liver and 66% for kidney, which is slightly greater than the amount of high complexity sequences found in poly(A)-containing mRNA (32% in liver and 45% in kidney). The somewhat higher saturation plateaus than determined by poly(A)-containing mRNA-driven hybridizations using cDNA probes may be due to the diversity of cell types and the presence of the non-poly(A)-containing mRNAs. Also, the assay for duplexes in the reaction mix was by hydroxylapatite chromatography rather than nuclease digestion (Britten et al., 1977). Therefore, the entire DNA molecule was scored as a duplex, thus elevating the level of DNA in the RNA hybrid. The saturation plateau with kidney mRNA is below that of liver, which is observed in other adult tissues (Galau et al., 1976; Hastie & Bishop, 1976).

From purified nuclei of rat liver, a group of DNA-binding NHP can be extracted by selective salt solubilization (Sevall et al., 1975; Kastraba & Wang, 1976). The DNA bound by these nonhistone proteins can be isolated, and analysis of its reassociation kinetics indicates that it is a subset of repetitive and single-copy sequences (Jagodzinski et al., 1979). The single-copy component of the bound DNA is 10% of the complexity of the whole single-copy component of the rat genome and has been used as a tracer in RNA-driven hybridizations. A 36.8% amount of the bound DNA is complementary to the liver mRNA driver. Thirty-four percent of the single-copy bound DNA component can hybridize with kidney mRNA. The rate of hybridization by the kidney mRNA is the same with both the single-copy bound tracer or the whole single-copy DNA. The rate observed for the liver mRNA hybridization with the nonhistone-bound single-copy DNA was threefold faster than that with the whole single-copy component of the rat genome.

The threefold rate increase for hybridization of rat liver mRNA and the rat liver nonhistone-bound single-copy DNA was tested for its significance by varying the rate of hybridization ($R_0t_{1/2}$) over a wide range and testing the quality of fit by measuring the goodness of fit parameter (Pearson et al., 1977). The best fit of the rat liver polysome hybridization data was $R_0t_{1/2} = 35.0$ M s ($k = 0.0198\ M^{-1}\ s^{-1}$) with a goodness of fit of 0.0156. Analysis of these data with fixed rates of $R_0t_{1/2} = 110$ M s ($k = 0.006\ M^{-1}\ s^{-1}$) and 11.7 M s ($k = 0.06\ M^{-1}\ s^{-1}$) yields goodness of fit parameters of 0.0250 and 0.0274, respectively. The goodness of fit obtained by fixing the rates varies by 64 and 77% from the best fit unfixed parameters when three experiments with different preparations of mRNA and bound DNA components were used. Likewise, the best fit rate for the kidney hybridization was $R_0t_{1/2} = 104$ M s ($k = 0.006\ M^{-1}\ s^{-1}$) with a goodness of fit of 0.0099. With fixed rates of $R_0t_{1/2} = 35.0$ M s ($k = 0.018\ M^{-1}\ s^{-1}$) and 385 M s ($k = 0.024\ M^{-1}\ s^{-1}$), the goodness of fit is 0.030 and 0.024, respectively. As for the best fit parameters for the liver hybridization, the kidney parameters are unique to the hybridization data. Thus, rat liver nonhistone proteins can concentrate a fraction of the rat genome which hybridizes more rapidly with rat liver mRNA.

In previous studies in mouse, cross-hybridization between cDNAs of individual complexity classes of liver poly(A)-containing message and kidney poly(A)-containing message indicated a substantial overlap between sequences transcribed in the two tissues. However, the low complexity cDNAs in liver cross-hybridized with the high complexity mRNA in kidney (Hastie & Bishop, 1976). The same is true for transcripts in chick oviduct and liver cells. That is, most transcripts are common between the two tissues, whereas differences do occur with respect to which complexity class the transcripts belong to (Axel et al., 1976). It has also been shown that it is the low complexity class of transcripts in developing myoblasts that gives rise to histospecific contractile proteins in myoblast cells (Paterson & Bishop, 1977).

Thus, the increased rate of hybridization of liver nonhistone-bound single-copy component with excess liver polysomal RNA may reflect the low complexity class of mRNA which is driving the hybridization. The percent mass of liver mRNA acting as driver in these reassociations is ~30%, which could represent the low complexity class of mRNAs. With kidney polysomal RNA, the rate of hybridization is that observed with the whole single-copy component, which represents the high complexity class of transcripts in the mRNA pool. In kidney polysomal RNA, the complementary transcripts to liver nonhistone-bound DNA are also represented in the high complexity class of mRNAs. This is consistent with the presence of liver nonhistones, which can increase the efficiency of specific groups of liver genes to be transcribed.

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Aminoacyl-tRNA Synthetase Stimulatory Factors and Inorganic Pyrophosphatase†

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ABSTRACT: A protein was purified from rat liver which stimulated a number of liver aminoacyl-tRNA synthetases. This stimulatory factor was identical with the "tRNA activator" of Dickman & Boll [(1976) *Biochemistry 15*, 3925] in its mechanism of action and chemical properties, although it was considerably more purified. The two preparations stimulated synthetases by virtue of their pyrophosphatase activity which destroyed the potent inhibitor, PP_i, that was present in the reaction mixtures. This PP_i was either generated during the reaction or was introduced by contamination of the tRNA or ATP preparations. The degree of inhibition of PP_i was strongly influenced by assay conditions, being most effective at low amino acid concentrations, at low pH, and in the presence of heterologous tRNAs. By use of certain assay conditions, PP_i concentrations as low as 2 μM could inhibit some synthetases close to 50%. The pitfalls associated with some assay conditions commonly used for aminoacyl-tRNA synthetases are discussed. These studies raise questions about the physiological significance of many previously described aminoacyl-tRNA synthetase stimulatory factors.

Since aminoacyl-tRNA synthetases catalyze the first committed step in a biosynthetic pathway, namely, protein synthesis, the possibility that the activity of these enzymes is subject to regulation must be considered. Over the years a number of laboratories have isolated factors which stimulate the activity of aminoacyl-tRNA synthetases and could thus serve some regulatory function. Boman & Svensson (1961) described a "regenerating enzyme" which increased the incorporation of methionine into *Escherichia coli* tRNA using yeast methionyl-tRNA synthetase. Makman & Cantoni (1966) isolated an "enhancing factor" which stimulated yeast seryl-tRNA synthetase when heterologous *E. coli* tRNA was the substrate, but which had little effect with yeast tRNA.

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