RNA TRANSCRIPTION FROM NONREPETITIVE DNA IN THE MOUSE

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SUMMARY:

Unique (nonrepetitive) DNA base sequences were isolated by hydroxyapatite chromatography from tritium-labeled mouse DNA. Total RNA purified from various tissues of adult mice was hybridized to the unique DNA, and DNA/RNA hybrids analysed on hydroxyapatite. Ten percent of the unique DNA was complimentary to brain RNA while less than two percent hybridized to RNA from liver, kidney and spleen.

INTRODUCTION:

Studies on the kinetics of DNA reassociation by Britten and Kohne have demonstrated that base frequencies in the mammalian genome range from highly repetitive to unique[1]. Nucleic acid hybridization techniques, as generally employed, assay RNA transcripts from repetitive DNA sequences[2]. Reactant concentrations and incubation times do not permit formation of unqiue DNA/RNA hybrids. In the mouse genome 70% of the total DNA sequences are unique[3]. This report presents the first analysis of RNA transcription from nonrepetitive DNA in mouse brain, liver, kidney and spleen tissue.

MATERIALS AND METHODS:

Exponentially growing mouse L cells were incubated in Basal Eagles Medium with 3 μC/ml ^{3}H-thymidine (New England Nuclear) for 72 hours. DNA of specific activity 9×10^{5} cpm/μg was isolated[4], sheared at 15,000 psi by passage through a French pressure cell, heat denatured and then renatured to a $C_{o}t$ of 220 in 0.12 M phosphate buffer pH 7 at 60°C.

C_0t is expressed as the logarithm of the product of the initial concentration in moles of nucleotides per liter (Co) and time in seconds (t)[1]. The DNA solution was diluted to give a phosphate buffer concentration of 0.03 M and loaded onto hydroxyapatite (HAP-BioRad) at 60°C. Double and single-stranded DNA were bound to the column. The phosphate buffer concentration was then increased to 0.12 M to elute DNA remaining single stranded after the incubation to C_0t 220. Kinetics of renaturation indicated that this DNA represented the unique or nonrepetitive base sequences of the mouse genome. Laird et al. have demonstrated that in the mouse genome repetitive DNA sequences renature prior to C_0t 220[3]. As is pointed out by McCarthy and Church the categorization of nucleic acid sequences as redundant or unique is somewhat arbitrary as it depends on the conditions of reassociation[2].

Total RNA was isolated from brain, liver, kidney and spleen of 6 - 8 week old Swiss white mice[5], treated twice with DNase I (Worthington)[4] and further purified by the method of Bellamy and Ralph[6]. RNA prepared in this manner eluted as a single peak from a Sephadex G-50 column[4]. Control experiments utilizing pulse labeled brain ^{3}H-RNA indicated minimal loss or breakdown during the purification procedure. Tritiated brain RNA was prepared from mice sacrificed 90 minutes after the intracranial injection of 100 µC ^{3}H-uridine (New England Nuclear) into each cerebral hemisphere.

Purified RNA was mixed with heat denatured unique ^{3}H-DNA (ratio 600 to 1) to give a final RNA concentration of 6 mg/ml in 0.12 M phosphate buffer. Aliquots of 50 µl were sealed in glass capillary tubes and incubated at 60°C. At specified intervals tubes were removed, their contents diluted to 0.03M phosphate buffer and loaded onto HAP columns (bed volume - 3 ml) at 60° C. Single stranded DNA and RNA were eluted with 0.12 M phosphate buffer and the column temperature

raised to 95°C to melt the DNA/RNA hybrid. The ratio of ^{3}H-DNA eluted at 95°C to total ^{3}H-DNA eluted was used in the calculation of the percentage of unique DNA hybridized to RNA. The fidelity of base pairing was determined by the thermal stability of the hybrid. No oligonucleotides were detected when control RNA samples, incubated along with the DNA/RNA hybrids, were run on Sephadex G-50 and 2.6% polyacrylamide gel electrophoresis[7]. Unique ^{3}H-DNA incubated without RNA for 120 hours did not self renature to a detectable extent at the assay concentration.

Contamination of RNA with small amounts of DNA might lead to the formation of DNA/DNA duplexes rather than DNA/RNA hybrids. As a control, RNA from the various tissues was incubated with RNase A (Worthington) at 50 µg/ml for one hour at 37°C. The nuclease was removed by two extractions with chloroform/octanol[4]. Samples of the RNase treated RNA preparations were incubated with unique ^{3}H-DNA. These controls indicate that 0.4% to 0.6% of the unique DNA hybridized to RNase treated RNA after 120 hours of incubation.

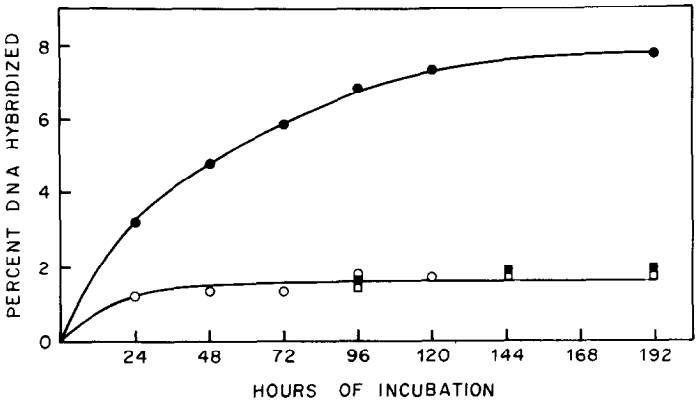

Figure 1. Hybridization of unique DNA sequences to total brain, liver, kidney and spleen RNA. Samples containing 0.5 µg of unique ^{3}H-DNA plus 300 µg of brain RNA (●), 300 µg of liver RNA (○), 300 µg of kidney RNA (□) or 300 µg of spleen RNA (■) in 50 µl of 0.12 M phosphate buffer were incubated at 60°C. The percentage of unique DNA hybridized to RNA at a given time was determined by chromatography on hydroxyapatite.

RESULTS:

The kinetics of the hybridization of unique DNA sequences to total RNA in 0.12 M phosphate buffer at 60°C are shown in Figure 1. These data are representative of values obtained from several RNA preparations isolated from each tissue . Liver, kidney and spleen RNA saturation of complimentary unique DNA sequences occurs much more readily than saturation with brain RNA. After 192 hours of incubation 7.8% of the unique hybridized with brain RNA while only 1.6% hybridized with liver RNA, 1.8% with kidney RNA and 1.9% with spleen RNA. Saturation of all complimentary unique DNA sites with brain RNA was not achieved by this time.

The reciprocal plot of data for brain RNA from Figure 1 is presented in Figure 2. A second-order dependence of hybridization is suggested by the linear relationship between the reciprocal of the amount of hybridization and the reciprocal of incubation time[8]. The intercept value indicates that 10% of the unique DNA would hybridize with brain RNA after an infinite period of incubation.

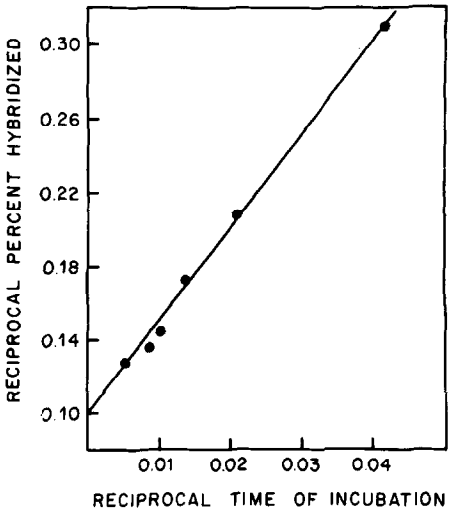

Figure 2. Reciprocal plot of the hybridization of unique DNA to total brain RNA. Data for brain RNA from Figure 1 was plotted according to the method of Bishop[8].

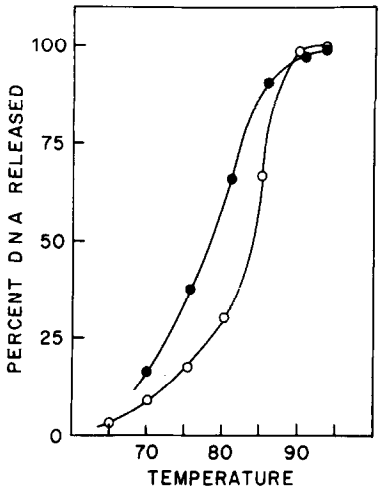

Figure 3. Melting profiles of unique DNA/RNA hybrid and unique DNA/DNA duplex. Samples of 0.5 µg of unique ^{3}H-DNA were incubated with 300 µg of total brain RNA (●) or 30 µg of sheared total DNA (o) for 120 hours as in figure 1. The percentage of ^{3}H-DNA released at increments of 5° C was determined.

The Tm of the unique DNA/brain RNA hybrid formed after 120 hours of incubation is 79°C (Figure 3). This compares favourably with the Tm of 84°C for the unique DNA/total DNA duplex formed under the same conditions (Figure 3). The sharp melting profile and high Tm indicates that few base pairs are mismatched in the DNA/RNA hybrid.

DISCUSSION:

Hybridization techniques, as generally employed, are limited to the assay of RNA transcripts complimentary to the repetitive DNA base sequences of the genomes of higher organisms[2]. Two exceptions have been reported. Davidson and Hough have isolated the unique DNA fraction of the Xenopus genome for use in hybridization to RNA purified from lampbrush stage oocytes[9]. The presence of enormous informational diversity in this RNA was demonstrated. RNA transcription from unique DNA sequences fractionated at C_0t 1500 in the mouse embryo has been reported by Gelderman, Rake and Britten[10]. Utilizing total embryo RNA they found that at least 8% of the unique DNA sequences

were transcribed. They also report that a large fraction of the rapidly labeled RNA from mouse embryo hybridized to unique DNA.

It is important to know whether unique genes contribute to RNA transcription during development. The theoretical complexity of transcription from unique genes based on DNA base sequence diversity is very great. The present study demonstrates that unique DNA fractionated at $C_o t$ 220 is involved in RNA transcription in various tissues of the mouse. It was found that 10% of the unique DNA hybridized to adult brain RNA while less than 2% was complimentary to adult liver, kidney or spleen RNA. The saturation values for liver, kidney and spleen RNA suggest that the extent of transcription of unique DNA sequences is quite similar in these tissues, however, it should be stressed that different unique DNA sequences may be transcribed.

This technique assays for total RNA transcripts present in the adult tissue which are complimentary to unique DNA sequences. RNA transcribed from these sequences at an earlier stage in development, and stored in a stable form could contribute to the total saturation value in the adult stage. The spectrum of RNA molecules present in adult brain includes transcripts from five times as many unique DNA sequences as in adult liver, kidney or spleen. The significance of the complexity of brain RNA, as revealed in this study, to higher neural function remains to be shown. If a correlation exists it would have interesting developmental and evolutionary implications.

ACKNOWLEDGEMENT:

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