HETEROGENEITY OF MOUSE UNIQUE DEOXYRIBONUCLEIC ACID

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1. Introduction

Kinetic analysis of renaturation has shown that mammalian DNA contains nucleotide sequences which differ in their renaturation rate. The most slowly reassociating component is thought to contain unique sequences, i.e. those present in only one copy in the haploid genome. The rate of renaturation of this DNA fraction in different species correlates with the size of the genome [1,2]. The renaturation follows second order kinetics and its course is rather similar to that of *E. coli* DNA, which does not contain repeated sequences [2]. The thermal stability and the hyperchromism of renatured DNA in this fraction are close to those of the native DNA [1,3]. On renaturation these sequences show a strong species specificity [3].

The proportion of DNA defined as unique may vary depending on the conditions of renaturation [4]. The following conditions are accepted as standard for isolation of mammalian unique DNA: fragment size of 200 to 500 base pairs (most often 400–500); incubation at 60°C in 0.12 – 0.14 M sodium phosphate buffer, pH 6.8 (NaP) and Cot > 200 [5,6]. Under these conditions the unique DNA in the mouse genome amounts to about 60% [1,7–9].

We found that a considerable heterogeneity can be observed in the unique DNA isolated under standard conditions, when allowing to reassociate at 50°C. The renatured DNA contained at least two fractions in approximately equal proportions differing in their thermal stability. T_m of the reassociated duplexes was 12°C below that of the fragmented mouse DNA for the first fraction and 2°C for the second one. The two fractions exhibited different hybridization ability with heterogeneous nuclear RNA and different thermal stability of the RNA–DNA hybrids obtained.

2. Materials and methods

2.1. *Isolation of DNA*

DNA from mouse liver and Ehrlich ascites tumour (EAT) cells was isolated as previously described [10]. The content of RNA and protein in the DNA preparations was below 1%. DNA (10 A_{260}-units/ml in 1 × SSC, specific radioactivity 5000 cpm/μg) was fragmented by sonication in an MSE ultrasonic power unit for 1 min at 1 A which gave fragments of 180–200 base pairs average length. After sonication the DNA samples were dialysed overnight against 50 mM NaCl, passed through a column of SE Sephadex C-25 (Na^+ form) to remove the bivalent cations, the solutions were made 0.2 M in NaCl and DNA was precipitated with 2.5 volumes of ethanol at −20°C.

2.2. *Separation of unique DNA*

The precipitated fragmented DNA was dissolved in 0.14 M NaP, denatured by heating in a boiling water bath for 15 min and allowed to reassociate at 60°C to Cot > 200. The basic experiments were performed with unique DNA isolated after reassociation to Cot = 500. The mixture was loaded on a column of hydroxyapatite (1 ml bed volume per 5–8 A_{260}-units of DNA, pre-equilibrated with 0.14 M NaP at 60°C) and the single stranded DNA was eluted with the same buffer at the same temperature. The eluate was dialysed overnight against distilled water, the solution was made 0.2 M in NaCl and the unique DNA was precipitated with 2.5 volumes of ethanol at −20°C.

2.3. *Renaturation and melting of unique DNA duplexes*

The precipitate of unique DNA was dissolved in 0.14 M NaP and the solution (5–6 mg/ml) was heated in a boiling water bath for 15 min. It was then incubat-

ed at 50°C to Cot = 10 000. After incubation the solution was diluted with 0.14 M NaP to a concentration of DNA of 5 A_{260}-units/ml and loaded on a hydroxyapatite column, pre-equilibrated with 0.14 M NaP at 50°C. The retained duplexes were melted by increasing the temperature in 2.5°C intervals and the single stranded DNA was eluted with 0.14 M NaP until $A_{260} < 0.01$.

For melting in solution, the renatured DNA was eluted from the hydroxyapatite column at 50°C with 0.48 M NaP, the solution was dialysed against 0.01 × SSC and brought to 1 × SSC. The melting was performed in an Unicam SP1800 spectrophotometer equipped with a heating attachment. Temperature was increased continuously at 1.5°C/min and the changes in A_{260} were recorded automatically.

2.4. *Isolation of heterogeneous nuclear RNA (HnRNA)*

HnRNA was isolated from EAT by thermal fractionation with phenol 0.14 M NaCl [11] The fractions extracted at 63°C and 85°C were purified from DNA and low molecular weight anionic contaminants as described previously [11].

2.5. *RNA:DNA hybridization with a vast excess of RNA*

The hybridization was carried out with highly labelled unique DNA and unlabelled 63°C and 85°C – HnRNA fractions, both isolated from EAT in a ratio 1:1000 at 65°C as described [7]. This kind of hybridization experiments require highly labelled DNA which is impossible to obtain from normal mouse tissue. As shown elsewhere [12] DNA of EAT does not differ from mouse DNA in reassociation and hybridization experiments. The RNA:DNA hybrids were melted on hydroxyapatite as described in 2.3. and the radioactivity of the effluents was measured in a Packard Tricarb 3320 scintillation spectrometer.

3. Results and discussion

The reassociation curve of mouse DNA (fig. 1) shows that at Cot > 200 renaturation of the unique DNA begins. In our experiments the unique DNA was isolated after incubation to Cot = 500. This was an additional precaution against possible contamination with non-renatured reiterated DNA. Because of the presence in this fraction of unique sequences only, it

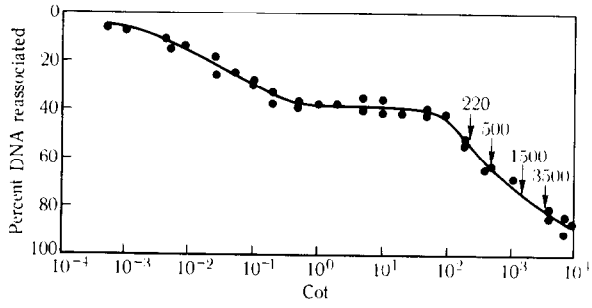

Fig. 1. Reassociation of mouse DNA. Reassociation carried out at 60°C in 0.14 M NaP, fragment length 180–200 base pairs. The curve is plotted according to Kohne and Britten [5]. The arrows indicate Cot to which the total DNA was allowed to reassociate before isolation of unique DNA.

should be expected that on renaturation perfect duplexes be formed. We carried out the renaturation of the unique DNA to Cot = 10 000 at a temperature 10°C below that of isolation. As shown in fig. 2, the duplexes formed under these conditions melted in a rather broad temperature interval, the T_m being 10°C lower than that of the native fragmented mouse DNA. Two transitions are present in the derivative melting curve the last of which begins above 80°C.

Since the renaturation was carried out under unusual experimental conditions (temperature of incubation 40°C lower than T_m of native DNA, moderate ionic strength) it might be asked whether the shape of the curve reflects a real heterogeneity of the renatured DNA or was an artifact of the conditions of incubation. To test this, unique DNA was separated preparatively into two fractions according to the thermal stability of the duplexes. The unique DNA was allowed to reassociate in 0.14 M NaP at 50°C to Cot 10 000 and then loaded on a column of hydroxyapatite at 50°C. The temperature was increased to 80°C and the melted duplexes were eluted with the same buffer. This fraction was designated as unique DNA I. The duplexes stable at 80°C were eluted with the same buffer at 98°C (unique DNA II). After dialysis and concentration, the fractions were allowed to reassociate under the same conditions. If these fractions were an artifact of the experimental conditions each one would give on reincubation the melting profile of the total unique DNA. However, fig. 3 shows. that this is not the case. Unique DNA I has a lower hyperchromism, melts in a broader temperature interval and its Tm is 66.5°C, i.e.

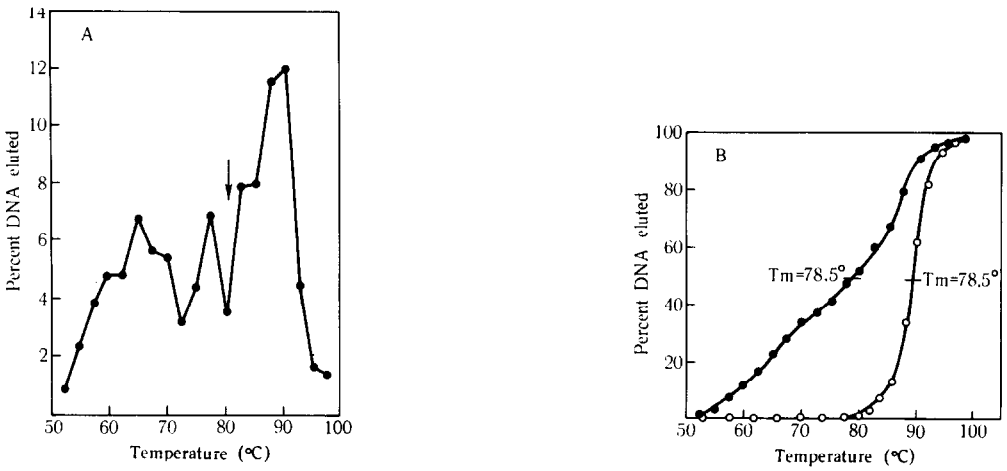

Fig. 2. Melting curves on hydroxyapatite of mouse unique DNA after reassociation to Cot = 10 000. A) Derivative melting curve. B) Integral melting curve. The open circles in B – melting curve on hydroxyapatite of fragmented native mouse DNA. The arrow in A indicates the temperature at which the unique DNA has been separated into two fractions.

12°C lower than T_m of the control native sheared DNA. Unique DNA II, however, forms almost perfect duplexes. Its hyperchromism is close to that of native DNA (32%) and is reached in a very narrow temperature interval. Its T_m is only 2°C lower than that of the control DNA. Therefore, under our experimental conditions the DNA sequences of the two unique fractions reassociate in a different way, perfect duplexes being formed only in unique DNA II.

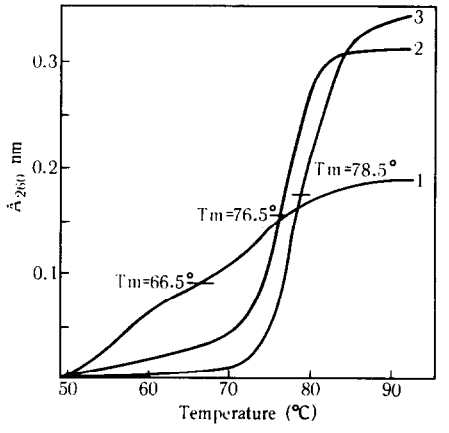

Fig. 3. Melting curves in solution of unique DNA I (1), unique DNA II (2) and fragmented native mouse DNA (3). Note that T_m on melting in solution (1 × SSC) is 10°C lower than that on melting on hydroxyapatite (0.14 M NaP).

The two unique DNA fractions were characterized also by their ability to hybridize with heterogeneous nuclear 63°C and 85°C RNA fractions isolated from EAT. As fig. 4 shows, the two DNA fractions exhibit different hybridization ability, higher in the case of unique DNA II. The thermal stability of the hybrids is also different (fig. 5). T_m's of the hybrids of unique DNA II with 63°C and 85°C RNA are 6–7°C higher than the corresponding T_m's of the hybrids of unique DNA I. The thermal stability of the hybrids unique DNA II-85°C RNA is even equal to that of the duplexes of unique DNA II.

These data show that using a lower temperature for reassociation of unique DNA, a real heterogeneity of this fraction may be disclosed. It should be noted that similar heterogeneity is found in unique DNA isolated after incubation to Cot 220, 1000, 1500 and 3500 (fig. 1). This rules out the possibility that the behaviour of the unique DNA is due to a contamination with repeated sequences. The heterogeneity of unique DNA is expressed in the different thermal stability of the duplexes formed. This suggests that the sequences in unique DNA I have a certain degree of similarity to each other. Although present as single copies in the genome, such sequences could hybridize and yield imperfect duplexes with a melting behaviour resembling that of reiterated DNA.

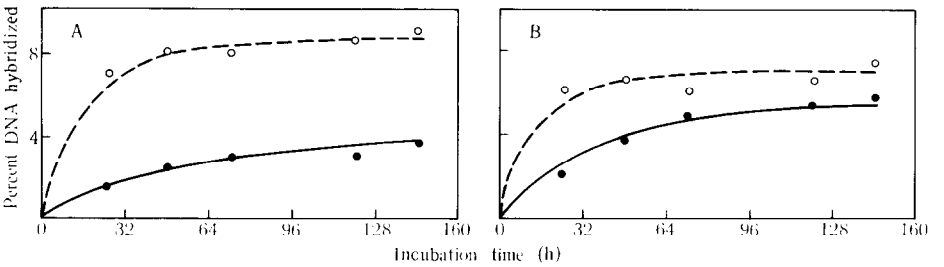

Fig. 4. Hybridization of unique DNA I and unique DNA II with vast excess of HnRNA. A) hybridization with 63°C–RNA; B) hybridization with 85°C–RNA; (o——o) – unique DNA I; (o——o) – unique DNA II.

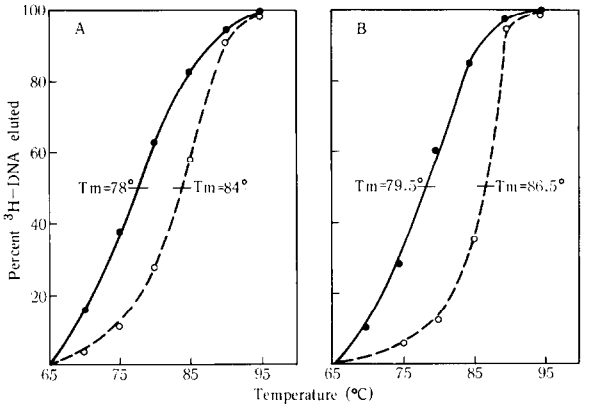

Fig. 5. Thermal stability of DNA:RNA hybrids. The symbols are as in fig. 4.

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