INTERSPERSION OF REPETITIVE SEQUENCES IN RAT LIVER DNA.

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In rat liver DNA, which contains only 20% repetitive sequences, a close interspersion of repetitive and unique sequences is found in about 35 % of the total DNA. The mean length of repetitive and unique alternating sequences is respectively 230 and 400 base pairs.

In the eukaryotic genome, the middle-repetitive sequences of DNA seem to be interspersed with unique sequences. This was demonstrated for Xenopus laevis (1), sea urchin (2, 3), calf thymus (4) and Drosophila (5) DNA's. On the other hand, unique and repetitive sequence transcripts are present in heterogeneous nuclear RNA (HnRNA) (6, 7, 8, 9). The average size of these sequences is about 150 to 400 base pairs, in both HnRNA and DNA (1, 2, 3, 5).

The general interspersion of repetitive and unique sequences in eukaryotic DNA, and the fact that the majority of repetitive RNA apparently never reaches the cytoplasm (10), suggest that these sequences play a regulatory role in transcription (11, 12).

We have studied the composition of rat liver DNA in order to extend these findings to DNA's with low repetitive sequence levels.

METHODS.

DNA purification was performed as in (13), except that phenol extractions were avoided to minimize the possibility of a loss of satellite fractions. DNA renaturation kinetics were done in 0,6 M NaCl, 10 mM Tris (pH 7,6) at 68°C, in a volume ranging from 500 to 50 µl, at a concentration varying from 0,2 to 5 mg/ml. The incubation periods varied from 7 sec to 49 h. Each tube contained 100 µg of DNA, with a mean length of 450 base pairs. After denaturation for 10 min at 100°C in 10 mM Tris (pH 7,6), DNA was rapidly cooled and 1/4 volume of 3 M NaCl, 10 mM Tris (pH 7,6) was added at 0°C. Before incubation the sealed tubes were heated for 10 sec at 90°C to reach quickly the incubation temperature. The incubation was stopped by freezing. When necessary the volume was quickly adjusted to 0,5 ml at 0°C by addition of cold 0,6 M

Abbreviation used: Cot = nucleotide concentration (moles/l) x incubation time (in sec).

NaCl, 10 mM Tris (pH 7,6). The percentage of renaturation was measured following two methods:

1°) nuclease_S1 digestion.

After thawing the tubes in ice, one volume of 10 % glycerol, 2 mM $ZnSO_4$, 0,06 M Na acetate (pH 4,6) was added, and incubation was performed during 1 h at 37°C with 2,5 µl of nuclease S1, prepared following the method of Vogt (14). Previous tests showed that, in those conditions (0,3 M NaCl; final pH = 4,5 at 20°C), nuclease S1 digests specifically single-stranded DNA, as already reported by others (14, 15, 16). The incubation was stopped at 0°C by addition of 1 ml of cold 10 % perchloric acid; the reaction mixture was then passed through nitrocellulose filters (Sartorius; pore diameter of 0,45 µ) presoaked and prefiltered with approximatively 20 ml of cold 5 % perchloric acid. The filters were rinsed with 0,5 ml of cold 5 % perchloric acid, and the filtrate (2,5 ml final) was recovered. The filters were soaked in 2,5 ml 5 % perchloric acid; filters and filtrates were then hydrolyzed during 30 min at 70°C. The percentage of DNA renaturation was estimated from the ratio between the absorption value at 267 nm of the filter fraction and that of the filtrate, taking as 100 % the sum of these values at the beginning of the renaturation. Corrections were made for hypochromicity in the last part of the curves.

2°) Hydroxyapatite_fractionation.

Hydroxyapatite (Bio-Rad; DNA-grade) was washed by successive decantations in 10 mM phosphate buffer (pH 6,8). 0,5 ml of wet paste were placed in 5 ml syringes stoppered with a Whatman GF/C glass fiber filter. Hydroxyapatite was then washed with about 20 ml of phosphate buffer at room temperature, then stored in 1 ml of this medium.

DNA (100 µg in 0,5 ml 0,6 M NaCl, 10 mM Tris (pH 7,6)) was diluted to 10 ml (final) of 10 mM Tris (pH 7,6) at 0°C, then loaded on the column at high speed (about 1 min) at 20°C. Denatured DNA was eluted by 5x1 ml of 0,16 M phosphate buffer at 60°C, then native DNA was recovered by elution with 5x1 ml of 0,5 M phosphate buffer at the same temperature. We have controled the efficiency of the separation, even for low molecular weight DNA. About 5 % of the denatured eukaryotic DNA is eluted as if it was native, even without incubation at 68°C; this is not the case with bacterial DNA, and must be due to the rapid self-annealing of "foldback" sequences (1).

RESULTS.

We have estimated the percentage and mean length of very repetitive, middle repetitive and unique sequences of rat liver DNA, by comparing the renaturation kinetics measured in two ways: nuclease S1 digestion and hydroxyapatite

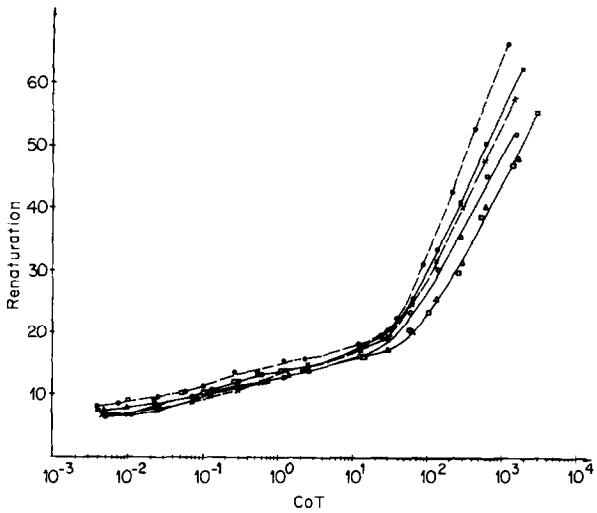

Fig. 1. Renaturation kinetics of rat liver DNA of different average sizes, measured by nuclease S1 digestion.
The mean numbers of base pairs were 1411 (⊙), 444 (×), 409 (●), 302 (⊘), 216 (○), 148 (▲) and 131 (□). These values were deduced from the sedimentation constants of DNA at 20°C in 0,1 N NaOH, 0,9 M NaCl, by Studier's relation (19). DNA was fragmented by sonication at 0°C, after saturation with He.

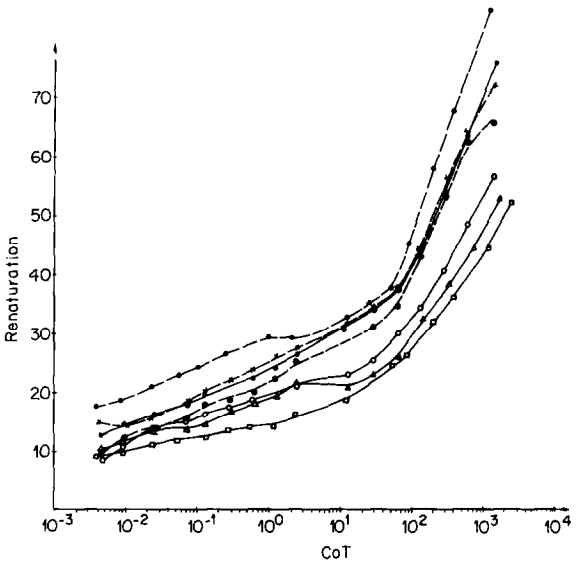

Fig. 2. Same legend as for fig. 1, except that DNA renaturation was measured by hydroxyapatite fractionation.

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fractionation. The principle is as follows: if unique or middle-repetitive sequences are located next to very repetitive sequences, large pieces of DNA should contain the two types of sequences covalently linked. On hydroxyapatite, the percentage of DNA renatured at a low Cot value would thus be overestimated. The measurement of this overestimate can be done by nuclease S1

digestion of single-stranded DNA. The difference between the renaturation percentages observed at each Cot value by the two methods, for different DNA sizes, is indicative of the respective distribution of unique and repetitive sequences in these DNA lengths. It is known that the renaturation kinetics depend on the DNA length (17); this fact does not interfere with our experiments, since the comparison between the two methods is performed for each DNA length and each Cot value in renaturation conditions which are absolutely identical.

Figure 1 shows the renaturation kinetics observed for DNA's of different lengths, as measured by nuclease S1 digestion. It is clear that the renaturation is speeded up when the DNA size is increased. On the other hand, the corresponding renaturation percentages estimated by hydroxyapatite fractionation are higher, indicating retention on the column of single-stranded DNA pieces covalently linked to reannealed duplexes of repetitive sequences (figure 2). The difference between the renaturation percentages measured by the two methods is plotted in figure 3, as a function of DNA size for different Cot values. At a Cot value of 25, one finds the presence of approximately 20 % repetitive sequences interspersed between unique sequences averaging 400 base pairs. For fragments 400 base pairs-long, 35 % of the genome (hydroxyapatite method) contains about 20 % of middle-repetitive sequences reannealed (S1 method) by Cot 25. Hence, the main size of middle-repetitive sequences can be calculated to be about 400 x 20/35 = 230 base pairs. Since, even at a DNA size of 1400 base pairs-long, only 35 % of the total genome binds to hydroxyapatite after renaturation to Cot 25, we conclude that approximately 65 % of the total DNA or 81 % of the unique sequences is

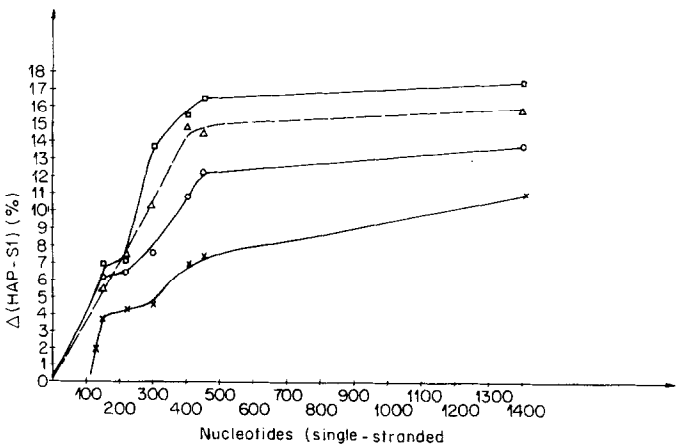

Fig. 3. Renaturation overestimate on hydroxyapatite, with respect to the renaturation values measured by nuclease S1 digestion.
(✕): Cot 0,01; (●): Cot 1; (▲): Cot 25; (■): Cot 100.

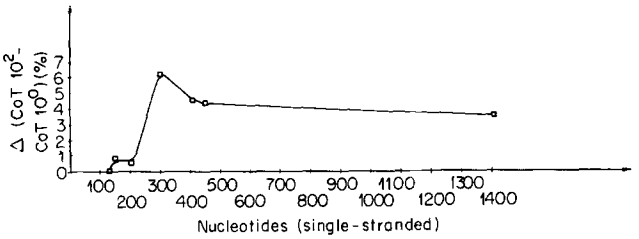

Fig. 4. A plot of the difference of renaturation overestimates by hydroxyapatite over S1 digestion between Cot 100 and Cot 1, as a function of DNA size.

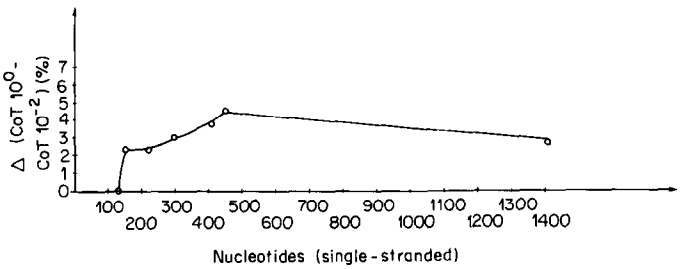

Fig. 5. Same legend as for fig. 4, between Cot 1 and Cot 0,01.

not arranged in a pattern of tight interspersion with repetitive sequences. Studies on Xenopus and sea urchin DNA (1, 2) have, in addition, indicated a longer period interspersion pattern, with 4000 base pairs long-unique sequences spacings. This type of arrangement is apparently absent in rat DNA, since the amount of DNA retained on hydroxyapatite in excess of the reannealed fraction measured by S1 digestion is at a plateau, for DNA sizes from 400 up to 1400 base pairs.

It is fair to assume that the overestimates given by the hydroxyapatite method over the S1 digestion method are mainly due to unique sequences, since these comprise 80 % of the rat genome. If one then substracts the overestimates between Cot values, the DNA size at which the difference is nil yields the length of the interspersed repetitive sequences. On the other hand, the difference reaches a plateau for a DNA size corresponding to the length of the interspersed unique sequences. For instance, the overestimate of hydroxyapatite over S1 digestion is the same at Cot 1 and 25 for fragments about 220 base pairs, in good agreement with the average length of repetitive sequences calculated above. When the data are analyzed in the same way between Cot 1 and 100 (figure 4), one finds that about 6,5 % of the total DNA consists of 220 base pairs long-repetitive sequences, interspersed with unique sequences at least 300 base pairs-long. On the other hand, the repetitive sequences renaturing between Cot 0,01 and 1 (figure 5) have an average

size of 130 and 300 base pairs, and are associated with unique sequences of 400 to 450 base pairs. This pattern of interspersion characterizes 5 % of the total DNA. Finally, the data for Cot below 0,01 (figure 3) indicate a length of about 130 base pairs for at least one fraction of the very repetitive sequences, which comprise probably the "foldback" sequences, and represent 7,5 % of total DNA. These sequences are associated with unique sequences of about 150, 400 and at least 1400 base pairs.

It can be added that no satellite DNA is detectable in rat liver, whether in CsCl, or in Cs_2SO_4-Ag^+ gradients. However, Roizès (18) has observed the existence in rat of several minor components having the properties of satellite DNA's, by treating DNA with restriction enzymes. The size of these "satellites" varies from 4000 to 400 base pairs. They must represent less than 3 % of total DNA, and be tightly associated with more complex sequences.

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