DISTRIBUTION OF REPETITIVE AND NON-REPETITIVE NUCLEOTIDE SEQUENCES IN THE DNA OF SEA URCHIN

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1. Introduction

It has been previously shown that the RNA populations synthesized in sea urchin embryos at early developmental stages are characterized by the high content of molecules complementary to repetitious nucleotide DNA sequences [1,2]. The functional role of these DNA sequences has not been established. It has been shown that some repetitive DNA sequences in the genome of the calf [3], the amphibian *Xenopus laevis* [4] and *Drosophila* [5] are distributed between non-repetitive nucleotide sequences. Both an arrangement is consistent with predictions from the gene regulation theory of Britten and Davidson [6].

This work describes the sequence organization of the sea urchin genome based on investigations of DNA renaturation kinetics. It is shown that the sea urchin genome contains a large fraction of repetitious DNA sequences of different degrees of repetition. The rest of the genome is represented by unique DNA sequences. About 25% of the whole genome is composed of continuous or rarely interrupted unique sequences. The comparison of reassociation kinetics of DNA fragmented to various extents revealed that a significant portion of the genome consists of alternating repetitious and unique nucleotide sequences. The results allow one to draw conclusions about the distribution in the genome of repetitious sequences varying in frequency.

2. Materials and methods

DNA was isolated from sperm of the sea urchin *Strongylocentrotus droebachiensis* by the phenol-detergent method [7]. RNAase and pronase-treated DNA was sheared ultrasonically into fragments of various lengths in a UZDN-1 sonic disintegrator. The size of a fragment was determined from the sedimentation coefficient ($S_{20,w}$) obtained in a Beckman Spinco E ultracentrifuge. In certain cases the molecular weights of DNA fragments were derived from their sedimentation rates in isokinetic sucrose density gradients in a Spinco L2-65K ultracentrifuge in the presence of an appropriately labeled DNA marker. DNA was denatured at 100°C for 10 min and annealed in 0.12 M phosphate buffer (pH 6.8) at 60°C. Denatured and reassociated DNA was separated according to the standard method of Britten [8] on hydroxyapatite columns (Biorad HT). The melting curves of native and reassociated DNA were obtained using a Jouan R-185 recording spectrophotometer or on an SF-4A spectrophotometer.

3. Results and discussion

The results of renaturation kinetic studies on sea urchin DNA fragmented to various lengths are presented in fig. 1 which shows the relationship between C_0t and the amount of reassociated DNA. The reassociation curve of *Escherichia coli* DNA is given for comparison. It is evident from the complex nature of the curves that sea urchin DNA is of considerable heterogeneity. By comparison of these curves with the renaturation kinetics of DNA from *E. coli*, carried out under identical conditions, the relative frequency of repetition of nucleotide sequences in individual DNA fractions may be obtained [8].

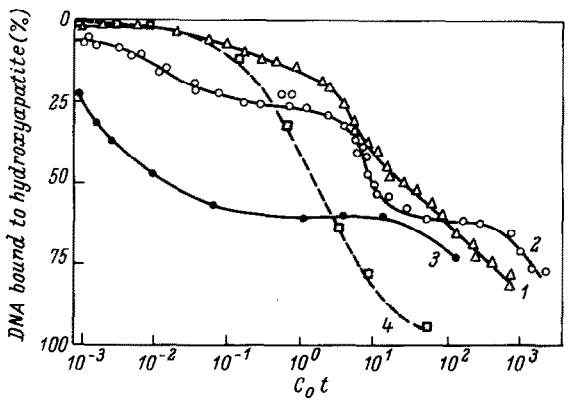

FIg. 1. The kinetics of reassociation of denatured DNA from the sea urchin *Strongylocentrotus droebachiensis*. DNA was sheared, denatured and incubated in 0.12 M phosphate buffer (pH 6.8) at 60°C. At various time periods DNA samples passed over a hydroxyapatite column at 60°C and the fraction of bound DNA was measured (ordinate). The DNA concentrations during the reassociation ranged from 5 μg/ml to 2mg/ml. Sizes of DNA fragments were: 1) 5 S, 300 nucleotide pairs; 2) 8 S, 700 nucleotide pairs; 3) 12.6 S, 3500 nucleotide pairs; 4) Reassociation of 700 nucleotidelong DNA fragments of *Escherichia coli*. Abscissa, $C_0 t$, the product of the initial concentration of DNA, C_0 (in moles of nucleotides per litre), and the time of incubation (in seconds).

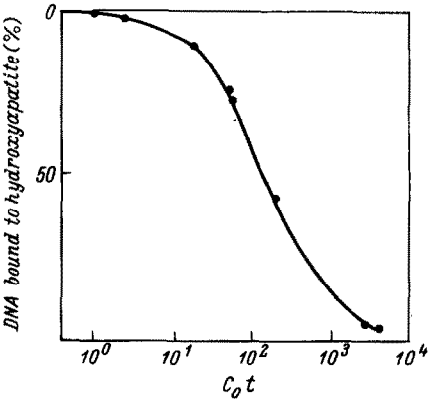

Fig. 2. Reassociation curve of non-repetitive fraction of sea urchin DNA. Repeated DNA sequences removed by previous hydroxyapatite fractionation after incubation of the 700 nucleotide-long fragments of DNA (8 S) at $C_0 t = 100$. Reassociation was performed in 0.24 M phosphate buffer (pH 6.8) at 60°C. Equivalent $C_0 t$ (abscissa) was calculated using a salt correction factor [3].

As seen from curve 1 for the 300 nucleotide-long DNA fragments only about 3% of the DNA reassociates rapidly at $C_0 t = 10^{-3}$. This fraction contains nucleotide sequences represented by a few thousand copies. DNA reassociating from $C_0 t = 10^{-3}$ to $C_0 t = 1$ contains about 15% of the whole DNA and represents huge repetitive nucleotide sequences with a hundred-fold redundancy. DNA fractions which make up 30% of the total DNA and reassociating from $C_0 t = 1$ to $C_0 t = 50–100$ represent intermediate and low repetitive sequences. Thus a considerable portion of the sea urchin genome consists of repetitious nucleotide sequences of DNA represented by fractions of different degrees of repetition.

Half the sea urchin DNA reassociates at $C_0 t$ about 40–50. All repetitive nucleotide sequences reassociates at $C_0 t = 100$. DNA fractions that do not reassociate at $C_0 t = 100$ are likely to represent predominantly unique sequences. The non-repetitive DNA fraction was separated from repetitive DNA on hydroxyapatite columns, after annealing at $C_0 t = 100$. It has been found that the kinetics of its renaturation are close to those of *E. coli* DNA (fig. 2). It is this fraction that we used in hybridization experiments to study transcription of non-repetitive DNA in the process of sea urchin development [9]. The melting profile of the single-copied DNA fraction unlike reassociated repetitive DNA (Fig. 3a) is similar to that of native fragmented DNA (fig. 3b). Therefore it may be suggested that this DNA fraction is represented exclusively by unique sequences.

The comparison of the reassociation kinetics of DNA fragments of various lengths enables us to approach the question of distribution of repetitive and unique sequences in the sea urchin genome as was done by Davidson et al. for *Xenopus* DNA [4]. It is evident that reassociation curves of DNA fragments of varying lengths differ drastically (fig. 1). With the increase of a fragment length the amount of reassociated DNA increases in the whole $C_0 t$ region corresponding to the renaturation of repetitive sequences. It is likely that DNA reassociated at low $C_0 t$ values represents duplexes of repetitious sequences to which 'tails' of unpaired unique sequences become attached. With increase of the fragment length these unpaired sequences grow longer.

In accordance with this suggestion the study of thermal stability of renaturation products shows that the increase of the length of DNA fragments results in

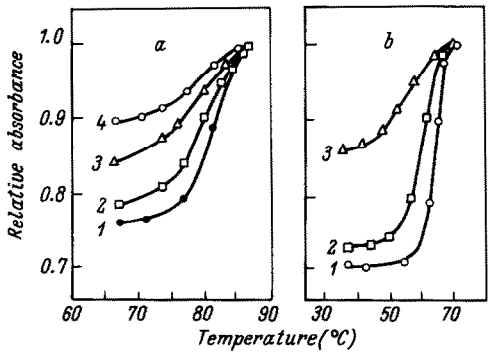

Fig. 3. Melting curves of reassociated sea urchin DNA. a) Dependence of melting profiles of reassociated at $C_0t = 100$ repetitive DNA on the length of DNA fragments. DNA was sheared to the 300 (1), 400 (2), 700 (3) and 3500 (4) nucleotide-long fragments. Melting was carried out in 0.12 M phosphate buffer. b) Comparison of melting profiles of native (1), reassociated at $C_0t = 5 \times 10^3$ non-repetitive DNA (2), and reassociated at $C_0t = 100$ repetitive DNA (3). DNA was sheared to the 700 nucleotide-long fragments (8 S). Melting was carried out in 0.03 M phosphate buffer (pH 6.8).

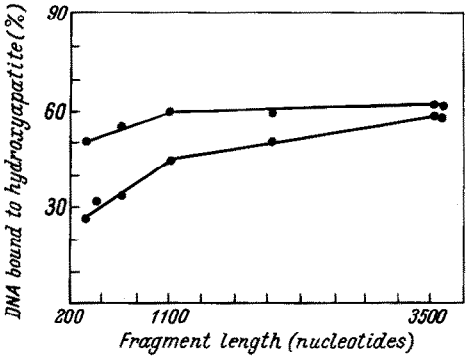

Fig. 4. Reassociation of the sea urchin DNA at $C_0t = 5$ (1) and $C_0t = 50$ (2) as a function of fragment length.

a significant reduction of hyperchromicity of the reassociated DNA although its melting temperature does not decrease much (fig. 3a). It may be concluded that a large portion of genome is composed of comparatively short repetitious sequences alternating with unique nucleotide sequences.

The average length of the repetitive and unique nucleotide sequences can be estimated from an analysis of the relationship between the size of a fragment and the degree of DNA renaturation at various C_0t (fig. 4). When the length of a fragment increases from about 300 nucleotide pairs (5 S) to 450 pairs (7.3 S), 700 (8 S) and then to 1100 pairs (9.6 S), the amount of DNA reassociated at low C_0t is markedly enhanced. At the same time, however, the hyperchromicity of reassociated DNA decreases, this decrease being about proportional to the fraction of reassociated DNA. Consequently, with the increase of the fragment length the number of nucleotides paired in the repetitious DNA duplexes remains almost unchanged in spite of the relative decrease of their content. Calculations based on the hyperchromicity data show that the average length of duplexes for DNA reassociated at $C_0t = 100$ is about 300 nucleotide pairs and is independent of the fragment length. Since the renaturation product of the smallest of the fragments we have investigated (300 nucleotides) has a distinct melting curve with hyperchromicity approaching to that of native DNA it can be assumed that the average length of repetitive DNA sequences is about 300 nucleotide pairs.

The length of unique sequences lying between the 300 nucleotide-long repetitive sequences is in any event no less than that of about 1000 nucleotide pairs since DNA reassociation enhances with the increase of the fragment length up to 1100 nucleotides. It is conceivable that on annealing of DNA fragmented to the length of about 1100 nucleotides all the non-repetitive sequences adjacent to repetitive ones are involved in reassociation. In fact, further increase of the fragment length to 1300–1400 and then to 2000 nucleotides is shown to result in a negligible increase of reassociation.

It should be noted, however, that the increase of DNA fragment up to 3500 nucleotide pairs (12.6 S) leads to a further increase of the degree of reassociation (at $C_0t = 10–100$). Had repetitious and unique sequences been distributed in genome only according to the aforementioned periodicity this should not have occured. Therefore, it may be suggested that there are longer unique sequences (of the order of 3500 nucleotides or even more) divided by short repetitious sequences. Besides, the sea urchin genome seems to contain long unique sequences over a great length not interrupted by repetitious sequences. In fact, even on renaturation of a very long fragment about 25–30% of DNA remains unreassociated at $C_0t = 100$. It is possible that sea urchin DNA also contains regions of compara-

tively long repetive sequences.

The analysis of the shape of reassociation curves gives information about the arrangement of DNA sequences among the family of repetitive sequences. Fig. 1 demonstrates that the shape of reassociation curves varies markedly for DNA fragments of different lengths. During renaturation of long fragments (curve 3) at small C_0t ($5 \times 10^{-2} - 10^{-1}$) the reassociation reaches 60%, i.e. it becomes almost identical to that at $C_0t = 10$. It is likely that highly repetitive DNA sequences renaturating at low C_0t involve in the association adjacent moderately and slowly reassociating repetitive sequences.

If the total length of one repetitive and one unique alternating DNA sequence makes up 1300–1500 nucleotides, a DNA fragment with the length more than 3000 nucleotides should contain at least two separate repetitive sequences. Since during renaturation of such fragments most repetitive sequences reassociate even at very low C_0t, one of the repetitive segments probably presents a highly repetitive nucleotide sequence. Hence, in the sea urchin genome repetitious sequences of various classes are organized in a definite order. The shape of reassociation curves at low C_0t value alters even when the length of a fragment increases from 300 to 700 nucleotides (fig. 1, curves 1 and 2). The 700 nucleotide-long fragments cannot contain two separate repetitive sequences divided by a unique portion. Therefore, it may be suggested that a part of the repetitive sequences of different frequency is clustered in a relatively long segment. Another possibility is that each of the 300 nucleotide-long segments of repetitive DNA contains short repetitive sequences of different degrees of redundancy.

This paper was being prepared when we received from Professor Eric Davidson a preprint of an article by Graham et al. [10] concerned with a study of interspertion of repetitive and non-repetitive nucleotide sequences in DNA of the sea urchin *Strongylocentrotus purpuratus*. When studying reassociation of labeled sheared DNA of different sizes with the excess of the 450 nucleotide-long fragments at $C_0t = 20$ the authors drew a similar conclusion regarding the general distribution of repetitive and unique nucleotide sequences in the sea urchin DNA.

We want to thank Professor Eric H. Davidson for acquainting us with this work before publication.

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