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Differently sized rDNA repeating units of Xenopus laevis are arranged as internally homogeneous clusters along the nucleolar organizer

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ABSTRACT

The organization of differently sized genes coding for rRNA in Xenopus laevis has been investigated by partial EcoRI or HindIII digestion. The electrophoretic patterns obtained revealed that most adjacent repeating units are equally sized. This conclusion is in agreement with a previous suggestion that the nucleolar organizer is made up of internally homogeneous blocks of rRNA genes.

INTRODUCTION

The length variability of the multiple copies of rRNA genes in Xenopus laevis (1) has been shown to be due to variations of the number of short subrepeats within the non transcribed spacer region (2).

Recently we have studied this variability in a population of Xenopus laevis (3); while a high degree of length polymorphism has been found in the population, each individual exhibited a few discrete classes of genes. As for the organization of differently sized genes along the nucleolar organizer, we found that equally sized genes are tandemly arranged. The method used was the examination at the electron microscope of reassociated high molecular weight rDNA. This conclusion was different from the one reached by other authors (4); they used a cloned spacer containing fragment as a probe for the analysis of the length of contiguous genes along the chromosomal rDNA and found them very often different in length.

Because of the relevance of this point for the understanding of gene rectification, that is of the mechanism(s) responsible for the parallel evolution of reiterated genes, this discrepancy needed further investigation by different approaches. The method used here is the electrophoretic analysis of fragments resulting from partial EcoRI or HindIII digestion of purified rDNA from Xenopus laevis individuals. This allows a direct investigation of adjacent genes with no involvement of intermediate steps such as cloning and reannealing procedure. The results obtained confirm that equally sized rDNA repeat units are mainly clusterd in blocks internally homogeneous.

MATERIALS AND METHODS

Extraction and purification of rDNA: Adult Xenopus laevis individuals were chosen with restriction patterns suitable for the analysis of partial digests (number, intensity and distribution of bands) (3). rDNA was extracted from erythrocytes and purified by two cycles of CsCl gradient centrifugation, as described previously (3). Care was taken to keep the molecular weight of the DNA as high as possible; in the various experiments it ranged from 40 to 70×10^6 daltons, as determined by analytical ultracentrifugation.

Partial digestion with restriction enzymes: 2 to 3 µg of purified rDNA in 0.5 ml of 0.05 M NaCl, 0.01 M $MgCl_2$, 0.1 M Tris-HCl (pH 7.5) were added with EcoRI and incubated at 37°C. The amount of the enzyme and the time of incubation were such to give a proper partial digestion. HindIII partial digestion was carried out in 0.05 M NaCl, 0.006 M $MgCl_2$, 0.006 M Tris-HCl (pH 7.5), at 37°C.

Gel electrophoresis: Partially digested rDNA was ethanol precipitated and redissolved in 100 µl of electrophoresis buffer (0.036 M Tris base, 0.001 M EDTA, 0.030 M NaH_2PO_4, pH 7.7) containing 8% (x/v) sucrose and 0.025% (w/v) bromophenol blue. The

samples were loaded on 33 cm x 1.1 cm, 0.3% (w/v) cylindrical agarose gels (BioRad) and run at 4°C at 2 V/cm for 24 hr and 32 hr when EcoRI and HindIII digests were respectively analyzed. Gels were stained, photographed and traced as described (3). Molecular weight scales were derived from EcoRI partial digests of λ DNA run in parallel and checked for expected properties of rDNA patterns; for instance, for prominent bands only, the size of the two-gene fragments had to be double with respect to the one-gene ones.

Computer simulation of partial digestion patterns: For each partial digestion to be carried out, the number of peaks resulting from a total digestion, their molecular weights and relative intensities were processed by a Fortran computing procedure which has been used to predict partial digestion patterns both in case of clustering and intermingling. By this procedure a vector containing either clustered or interspersed gene sequences has been generated, from which new vectors were derived according to the molecular weights of single fragments and degree of digestion. The number of rRNA gene was set to 1000 per genome. Intermingling of differently sized repeat units has been obtained by a specific routine of randomization (5) which supplied random numbers in an interval defined by the number of peaks following complete digestion.

RESULTS AND DISCUSSION

EcoRI partial digestion: EcoRI makes two cuts per rDNA repeating unit, producing two types of $\frac{1}{2}$-mers, a homogeneous fragment of 3×10^6 daltons (A in Figure 1), and one heterogeneous in length (B in Figure 1) which contains the untranscribed spacer region. Gel electrophoretic analysis resolves the heterogeneous (B) fragments in a pattern of bands whose number, molecular weights and relative intensities are characteristic for each individual (3). Beside the above described $\frac{1}{2}$-mers, partial

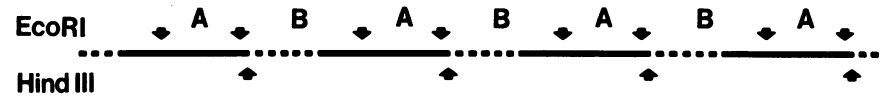

Figure 1. EcoRI and HindIII restriction sites in rRNA genes of Xenopus laevis. (———) transcribed region; (------) untranscribed spacer region. A and B are the constant and the variable fragments respectively resulting from EcoRI total digestion.

EcoRI digestion is expected to produce 1-mers, that is the complete repeating units, when one EcoRI site is missed (AB or BA in Figure 1), $1\frac{1}{2}$-mers when two contiguous sites are missed (ABA or BAB), 2-mers (ABAB or BABA) and so on. The presence of two B regions in the same fragment allows the analysis of the length of adjacent genes. Actually the analysis has been limited up to 2-mers due to the unsatisfactory resolving power of gel electrophoresis for fragments of higher molecular weight.

Obviously the 1-mers are expected to reproduce faithfully the pattern of the heterogeneous $\frac{1}{2}$-mers both in case of clustering and intermingling of differently sized genes. On the contrary the pattern of the $1\frac{1}{2}$-mers and 2-mers are expected to distinguish between the two possible alternatives. In fact in case of clustering the pattern of 2-mers would also reproduce the one of B type $\frac{1}{2}$-mers, while in case of intermingling additional bands would complicate the pattern because of the contiguity of genes of different length, in various combinations. Moreover the pattern of $1\frac{1}{2}$-mers is expected in any case to be more complicated. Namely when two contiguous EcoRI sites are missed, two types of $1\frac{1}{2}$-mers are produced: ABA and BAB in Figure 1. Of these only the bands corresponding to BAB fragments permit to discriminate between clustering and intermingling. The use of the computer has been instrumental to predict, from the data of total digestion, the results of partial digestion, both in case of clustering and intermingling.

Figure 2a shows the photograph of a gel of partially EcoRI

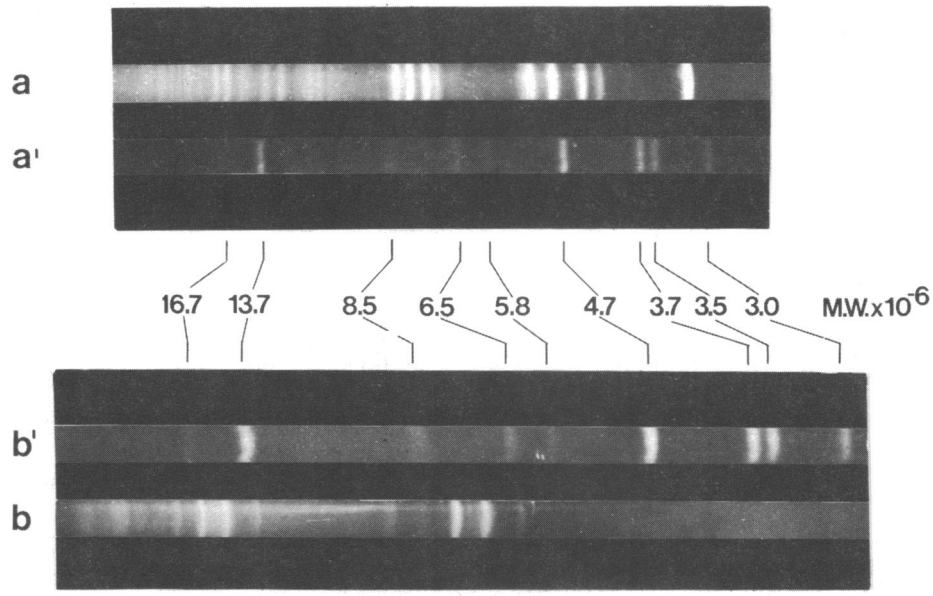

Figure 2. Gel electrophoresis of rDNA from two individuals, partially digested with (a) EcoRI and (b) HindIII; a' and b' are parallel runs of EcoRI partial digests of λDNA.

digested rDNA from a male Xenopus laevis with the two nucleoli very different in size, that is with most rRNA genes probably located on one of the two homologous nucleolar organizers. The densitometric tracing of this gel (Figure 3c) reveals that the rDNA fragments are resolved as discrete bands up to molecular weight values of about 17 x 10^6 daltons, corresponding to two genes. In the same Figure the gel densitometric tracing is compared with computer predictions following clustering (Figure 3b) and intermingling (Figure 3a). From this comparison the strict similarity of the experimental profile with the one predicted for clustered organization of differently sized genes is evident; the relative amounts of $\frac{1}{2}$-mers, 1-mers, $1\frac{1}{2}$-mers and 2-mers, which depend on the extent of digestion, is obviously not relevant. The computer prediction in case of intermingling reported in Figure 3, is only one example of the many tested random assortements of differently sized genes. Moreover computer simulated partial

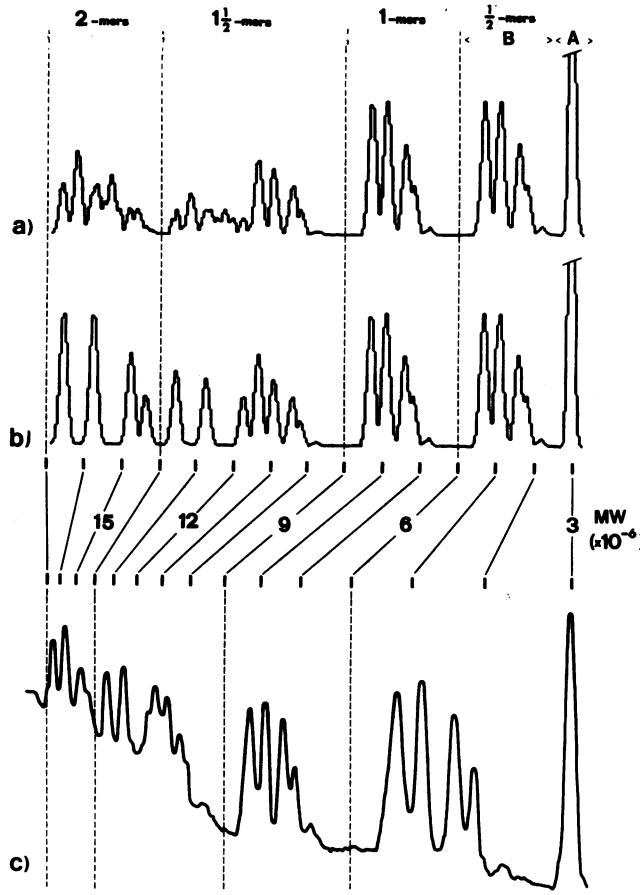

Figure 3. Theoretical and experimental electrophoretic patterns of partially EcoRI digested individual rDNA. (a) Computer simulated profile of partial digestion in case of random intermingling of genes of different size classes; (b) Computer simulated profile obtained when equal repeats are set adjacent to each other; (c) Densitometric tracing of gel photograph.

digestions have been carried out (here not shown) also according to other criteria of gene arrangement: for example the genes of different length have been separately intermingled in two groups, in various combinations. The pattern predicted for clustering was the one which best reproduced the experimental profile.

A similar analysis has been carried out for the rDNAs from two females, both with unequally sized nucleoli; in these cases again, the experimental patterns resembled closely those predic-

ted by the computer for a clustered arrangement of equally sized repeating units (data not shown).

HindIII partial digestion: HindIII makes only a single cut per rDNA repeating unit (Figure 1) generating a pattern of fragments of different length. Partial digestion is expected to produce only entire multiples of the repeating unit. The rationale of this experiment is the same of the one above described for EcoRI, except for the absence of $\frac{1}{2}$-mers and $1\frac{1}{2}$-mers. Figure 2b and 4a show the photograph and the densitometric tracing of the fragments resulting from a partial HindIII digestion of rDNA from a female with equally sized nucleoli. Figure 4b gives the result of a similar analysis carried out on rDNA from another female with two different nucleoli. It is evident that the pattern of 2-mers reproduces in both cases the one of 1-mers.

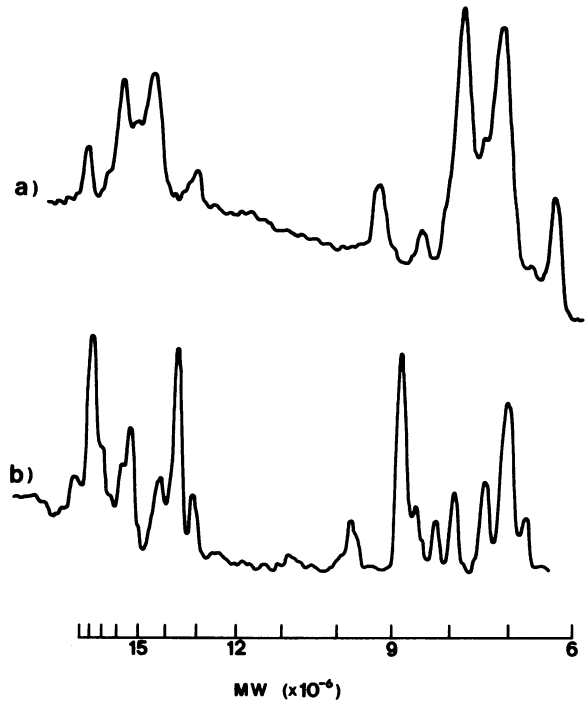

Figure 4. Densitometric tracings of gel photographs of two individual rDNAs partially digested with HindIII.

CONCLUSIONS

Both from EcoRI and HindIII partial digestion experiments the conclusion can be drawn that most contiguous rDNA repeating units are equally sized. In other words, rRNA genes are organized in blocks internally homogeneous. This conclusion derives from the analysis of a total of eleven different individuals, six in the previous (3) and five in the present paper, analyzed by two different techniques. However these data cannot exclude the possibility that in the animals analyzed a low degree of intermingling might be present, below the limits of the techniques used. Moreover, the existence of some occasional individuals with a high degree of intermingling among differently sized genes, cannot be excluded either, because of the small sample of animals examined.

The main interest of our conclusion resides in its implications on the problem of the parallel evolution of reiterated genes. Several models have been proposed in order to explain this peculiar evolutive behaviour (6-13). The evidences presented in our previous (3) and in the present paper, namely that most equally sized genes are clustered in homogeneous blocks, are difficult to reconcile with one of the proposed models (9-13) which assigns to unequal sister chromatid exchanges a relevant role in rRNA genes parallel evolution.

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