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Short Communication

Characterization of ribosomal DNA (rDNA) in *Drosophila arizonae*

Francisco Javier Tovar[1], Luiz Antônio Ferreira da Silva[2], Renato Santos Rodarte[1] and Orilio Leoncini[1]

Abstract

Ribosomal DNA (rDNA) is a multigenic family composed of one or more clusters of repeating units (RU). Each unit consists of highly conserved sequences codifying 18S, 5.8S and 28S rRNA genes intercalated with poorly conserved regulatory sequences between species. In this work, we analyzed the rDNA of *Drosophila arizonae*, a member of the *mulleri* complex (Repleta group). Using genomic restriction patterns, cloning and mapping of some representative rDNA fragments, we were able to construct a representative restriction map. RU in this species are 13.5-14 kb long, restriction sites are completely conserved compared with other drosophilids and the rDNA has an R1 retrotransposable element in some RU. We were unable to detect R2 elements in this species.

INTRODUCTION

In eukaryotes, ribosomal DNA (rDNA) is a multigene family composed of repeating units (RU). The genus *Drosophila* rDNA has been extensively analyzed specially in *Drosophila melanogaster*. In the case of *D. arizonae*, RU are clustered in the X chromosome nucleolar organizer and in a secondary nucleolar organizer present in a minichromosome (Bicudo and Richardson, 1977; Bicudo, 1981).

Each RU consists of a gene region for 18S, 5.8S, 2S and 28S rRNA separated by internal transcribed spacers (ITS), and an intergenic spacer (IGS) separating two gene regions. In *Drosophila* as well as in other insects, some RU are interrupted by retrotransposable elements (Jakubczak *et al.*, 1990).

In the present study we characterize molecularly the rDNA of *D. arizonae*, a Mulleri complex member, by establishing rDNA restriction patterns and cloning representative *Eco*RI rDNA fragments. Partial sequencing of these clones made possible retrotransposon identification. Results were used to construct rDNA maps of RU.

MATERIAL AND METHODS

Specimen: *Drosophila arizonae* stock derived from a pool of 25 isofemale lines, collected in Guayalejo, Mexico, was kindly provided by H.E.M.C. Bicudo.

DNA isolation: Genomic high molecular weight DNA was isolated following Ish-Horowicz (1989).

λ DNA isolation: Bacteriophage particles were purified according to Yamamoto *et al*. (1970) in combination with a glycerol step gradient (Vande Woude *et al*., 1979). Phage DNA was isolated according to Sambrook *et al*. (1989).

Plasmid DNA isolation: pUC19 DNA isolation was carried out using a "FlexiPrep" kit (Pharmacia Biotech).

Restriction enzymes: We used *Eco*RI (BRL), *Sma*I (BRL), *Hin*dIII (BRL), *Pvu*II (New England Biolabs), *Hin*cII (New England Biolabs), *Xba*I (Boehringer Mannheim) and *Pst*I (BRL). Reaction procedures followed supplier instructions.

Molecular cloning: *D. arizonae* genomic DNA was totally digested with *Eco*RI and ligated into λEMBL-4 at *Eco*RI sites using T4 ligase. Ligation was carried out at a 5:1 ratio of vector to genomic DNA.

In vitro packaging of DNA: We prepared packaging extracts from one lysogen (*Escherichia coli* SMR10) according to Sambrook *et al*. (1989). For recombinant phage selection we used *E. coli* Q359, while Q358 was used for amplifying selected clones.

Screening and subcloning: Screening was carried out as described by Benton and Davis (1977), using a probe radiolabelled pDm238 containing an rDNA repeat unit from *D. melanogaster* (Rohia *et al*., 1981). Fragments of rDNA from positive amplified phages were subcloned into the *Eco*RI site of pUC 19.

Sequencing: *pDarz3.9, pDarz4.5, pDarz8.0* and *pDarz15* clones were partially sequenced using a T7 Sequencing Kit (Pharmacia). Sequences were aligned manually. We used forward and reverse universal primers for pUC and 28Sins1 (5' AGCGGGTGTTGACACAATGTGATTTC 3') primer.

Labelling of DNA: The DNA was labelled by the nick translation procedure (Nick Translation System, BRL) using [α-^{32}P]-dATP as radioactive nucleotide.

[1]*Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Caixa Postal 68011, 21944-970 Rio de Janeiro, RJ, Brasil. Send correspondence to O.L. E-mail: leoncini@biologia.ufrj.br*
[2]*Departamento de Biologia, Centro de Ciências Biológicas, Universidade Federal de Alagoas, AL, Brasil.*

RESULTS

Analysis of rDNA restriction patterns

High molecular weight DNA was isolated and digested with *Eco*RI, *Sma*I, *Pst*I, *Xba*I, *Pvu*II, *Hin*dIII and *Hin*cII restriction enzymes to establish rDNA restriction patterns, digested DNAs were separated by electrophoresis in agarose gels, transferred to nylon membranes and hybridized to *pDm238*.

Cloning of *Eco*RI representative fragments

To clone rDNA fragments, we digested genomic DNA and λEMBL-4 DNA using *Eco*RI endonuclease. Digested DNAs were mixed in different ratios and ligated using T4 DNA ligase (Pharmacia). Ligated DNA was packaged *in vitro* and recombinant λ clones were selected using *E. coli* Q359. Clones carrying rDNA were selected by hybridization using *pDm238* radiollabeled probe. Selected λ clone rDNA fragments were subcloned into pUC19 plasmid. The rDNA restriction patterns together with data from clones were used to construct maps presented in Figure 1.

Identification of retrotransposable elements

To identify transposable elements present in this fly we sequenced junction regions (28S-transposon) from several clones. The presence of R1 retrotransposons became evident when using the insertion point as the identification criterion. In addition, we sequenced internal regions in putative R1 DNA and found similarities with *gag* and *pol* ORFs of R1 from *D. mercatorum* and *D. hydei* (GenBank, accession numbers AF114255, AF114256 and AF114257).

DISCUSSION

Analysis of restriction patterns and physical maps clearly shows heterogenic rDNA structure, a fact already observed in other rDNAs from genus *Drosophila*. This heterogeneity is due to a small intergenic spacer length polymorphism and the presence (in a number of repeating units) of R1 transposable elements. Genomic DNA digested by *Eco*RI and hybridized with *pDm238* shows bands of 13.5-14 kb (complete RU), 8.2 kb (internal transcribed spacer, 28Sα gene and 5' end of R1 transposable element) and 6.5 kb (3' end of RI transposable element, 28Sβ and part of the intergenic spacer). In addition to these bands, other weak bands appear with lengths between 3.0 to 4.5 kb, all showing the same structure of that of 6.5 kb described above.

In *D. arizonae* rDNA, repeating units (non-interrupted) had a mean value of 13.5-14 kb in length, slighty longer than in *D. melanogaster* due to an increase of the intergenic spacer length. Internal transcribed spacers are conserved in length compared to *D. melanogaster*. Restriction sites present in coding regions are conserved as in other drosophilids. We also found a polymorphic *Eco*RI site in the IGS near (~1 kb) 3' 28S gene.

According to our data, R1 elements in this species are 6 kb long. We were unable to detect the presence of R2 elements due to lack of a specific probe; probably this species has R2 elements in low numbers (other members of the Repleta group carry it) but we have no evidence of that.

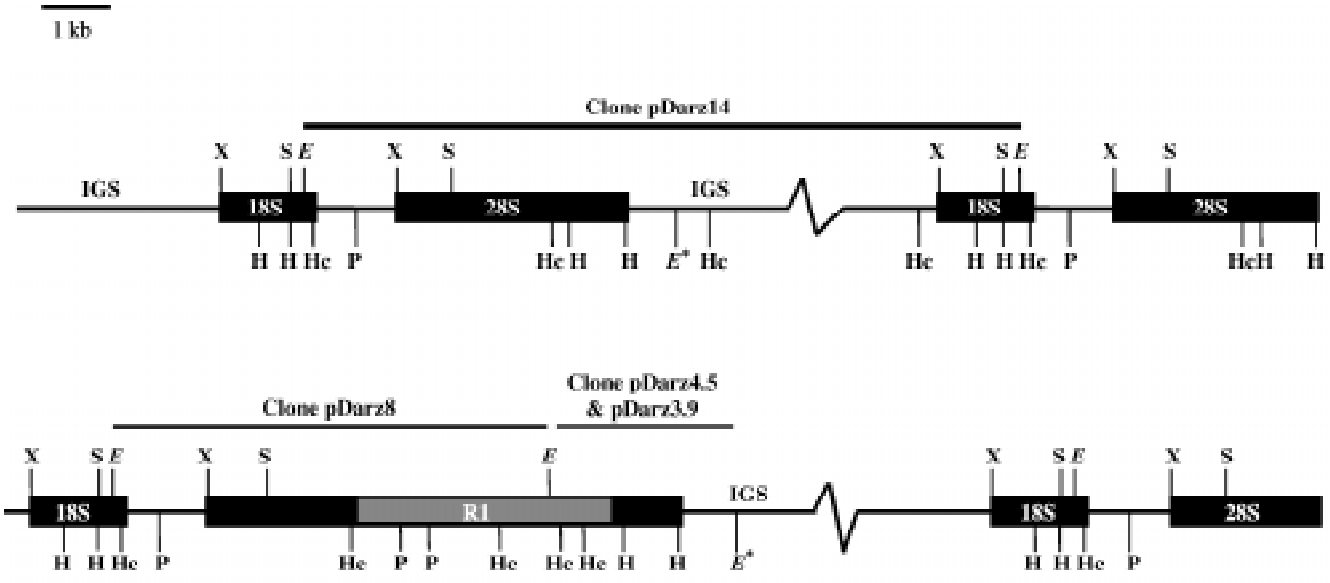

Figure 1 - rDNA restriction maps proposed for *Drosophila arizonae*. IGS, Intergenic spacer; *E*, *Eco*RI; *E**, *Eco*RI polymorphic site; H, *Hin*dIII; Hc, *Hin*cII; P, *Pst*I; S, *Sma*I and X, *Xba*I.

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RESUMO

O DNA ribossômico (rDNA) é uma família multigênica composta de um ou mais aglomerados de unidades de repetição (RU). Cada unidade consiste de seqüências altamente conservadas que codificam os rRNAs 18S, 5.8S e 28S, intercaladas com seqüências regulatórias pouco conservadas entre as espécies. Neste trabalho analisamos o rDNA de *Drosophila arizonae*, um membro do complexo *mulleri* (grupo Repleta). Usando padrões de restrição genômicos, clonagem e mapeamento de alguns fragmentos de rDNA representativos, estabelecemos um mapa de restrição do rDNA representativo desta espécie. Neste drosofilídeo, a RU tem um tamanho médio de 13.5-14 kb e os sítios de restrição estão completamente conservados com relação a outras drosófilas. Além disto, este rDNA possui um elemento transponível tipo R1 presente em algumas unidades. Neste trabalho não tivemos evidências da presença de elementos R2 no rDNA desta espécie.

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