MOLECULAR-GENETIC CHARACTERISTICS OF LIZARD RIBOSOMAL DNA

A. S. Voronov,[1] G. A. Voronova,[1]
N. S. Kupriyanova,[1] and A. P. Ryskov[1]

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INTRODUCTION

Remarkable changes in sequence composition of different genome components took place in the process of transition between cold- and warm-blooded vertebrates. The possible way to study this transition on the molecular level today is to compare mammalian or avian DNA sequences with homologous sequences in *Xenopus*, the only cold-blooded vertebrate having enough sequences in data banks. Ribosomal DNAs (rDNAs), and particularly internal transcribing rDNA spacers (ITS-1 and ITS-2), are commonly used in comparative genomic studies of different taxa providing valuable data for phylogenetic and systematic constructions. Unfortunately, information on sequence organization of rDNA in reptiles is also very limited. Here, we present first data on sequence organization of rDNA monomers and its ITS-1 and ITS-2 regions for several lizard species.

MATERIAL AND METHODS

Darevski nairensis, *Darevski valentini*, *Lacerta strigata*, and *Laudakia caucasia* total genomic DNAs were isolated from the lizard blood samples, according to Mathew (1984). Two oligonucleotide primers 5′-AGTCCCTGCCCTTTGTACACA-3′ and 5′-GCCGCGTCTGATCTGAGGTC-3′ were designed and used for PCR amplification of the rDNA segment, containing ITS-1 and ITS-2. The oligonucleotide probes RI, RII, RIII, and RIV used in blot-hybridization experiments were the same as described earlier (Kupriyanova et al, 1996). PCR amplification and blot hybridization were carried out as previously described (Kupriyanova et al., 1999). Comparison between 3′18S-ITS-1-5.8S-ITS-2 regions of the *L. strigata*, *Xenopus laevis* and *Rattus norvegicus* was performed after their alignment with the use of "DNA Star" program (Clustal).

[1] Institute of Gene Biology, Russian Academy of Sciences, Vavilov str. 34/5, 119334 Moscow, Russia; E-mail: ryskov@mail.ru.

RESULTS AND DISCUSSION

Figures 1 and 2 show restriction maps and blot-hybridization patterns obtained for *D. nairensis* and *Laudakia caucasia* lizard rDNAs. Hybridizations were carried out with the use of oligonucletide probes complementary to the most conservative transcribed regions of rDNA and different restriction enzymes. The conservative EcoR I sites characteristic of other vertebrate 18S and 28S rDNAs are found in all lizards studied. Positions of some other restriction sites are similar for *D. nairensis* and *D. valentini* rDNA, but differ for rDNA of *Laudakia caucasia*. The blot-hybridization data obtained allow to conclude that rDNA monomers in *D. nairensis* and *D. valentini* are about 15 – 16 kb and rDNA monomers in *Laudakia caucasia* are about 10 kb in length.

To study rDNA regions containing 3′ end of 18S rDNA, ITS-1, 5.8S rDNA, and ITS-2 in different lizard species, PCR amplification with primers to conservative 18S and 28S rDNA sequences was used. Here, only completed data for *L. strigata* are presented. The amplified 2 kb DNA fragment of *D. strigata* was cloned into T-Easy vector (Fermentas), sequenced and compared with homologous segments of *Xenopus laevis* and *Rattus norvegicus* rDNAs (Fig. 3). Our calculations showed that total percentage of similarity of 3′-18S, 5.8 rDNA, and ITS-1 + ITS-2 regions between these organisms is about 89, 94, and 16.5%, respectively. It is interesting that the great bulk of the coincident nucleotides in ITS-1 + ITS-2 is "G" and "C" whereas only 6 coincident "A" and 4 coincident "T" are detected among 302 identical positions in ITS regions. Surprisingly, but similarity between ITS regions of *L. strigata* and *Rattus norvegicus* comprises 45% and this is much higher than pairwise similarity between ITS regions of *L. strigata*/*Xenopus laevis* and *Xenopus laevis*/*Rattus norvegicus* that comprise 21 and 24% respectively. Of course, rDNA sequence information for other lizard species is necessary to make evolutionary comparisons, and this work is now in progress.

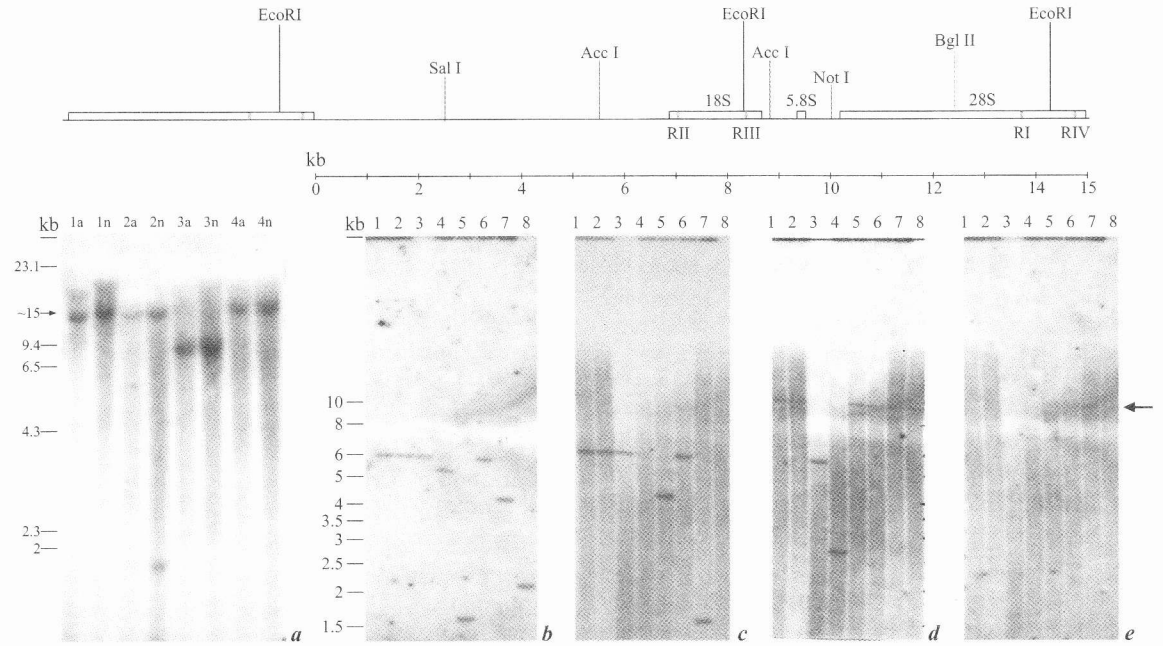

Fig. 1. Organization of a 15-kb repeating ribosomal DNA unit of rock lizards. Identical results were obtained for *D. nairensis* and *D. valentini* lizards. *a*: *D. nairensis* lizard DNA after digestion with HindIII (1); BamHI (2); EcoRI (3); KpnI (4) was blot-hybridized with labelled oligonucleotide RII. Identical sizes of the hybridizing fragments on lanes 1a – 1n, 2a – 2n, and 4a – 4n let to conclude that putative length of repeating rDNA unit in rock lizards approximates to 15 kb (shown by the arrow). *b* – *e*: Digestion of *D. nairensis* genomic DNA with EcoRI (1); EcoRI + Pst I (2); EcoRI + SalGI (3); EcoRI + AccI (4); EcoRI + BglII (5); EcoRI + EcoRV (6), EcoRI + Not I (7); EcoRI + Xho I (8) and successive blot-hybridization with RI (*b*), RIII (*c*), RII (*d*), or RIV (*e*). Positions of the probes used for hybridization are shown on the rDNA restriction map above. The right arrow in (*e*) shows location of the 10 kb hybridizing fragments.

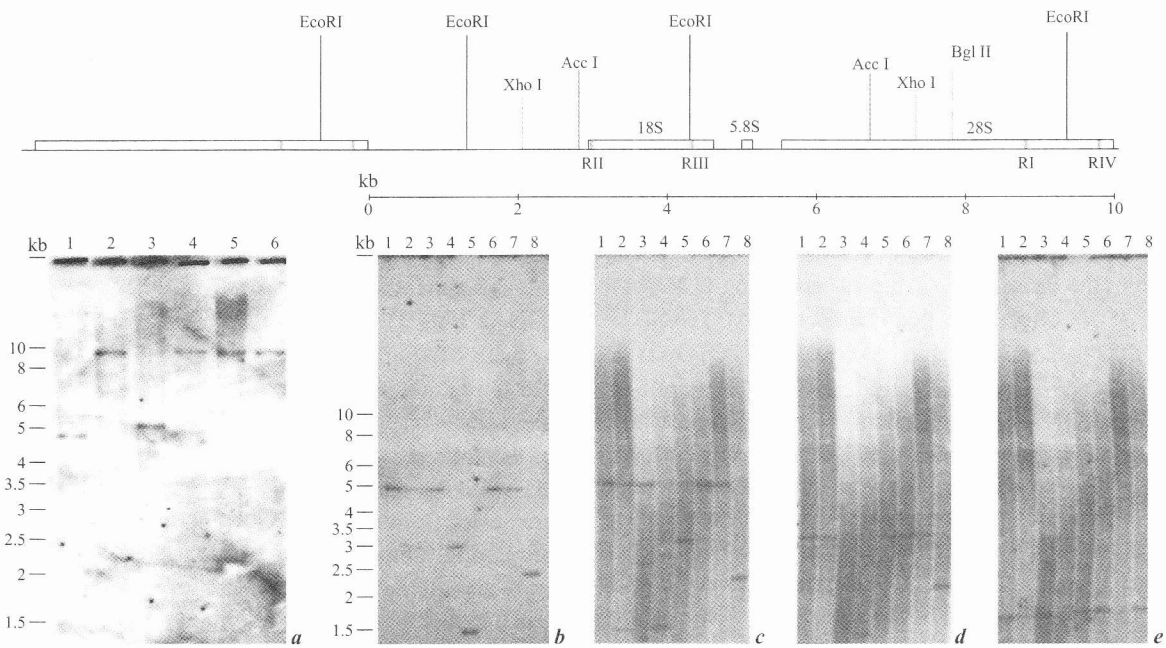

Fig. 2. Organization of a 10 kb repeating ribosomal DNA unit of *Laudakia caucasia*. *a*: *Laudakia caucasia* DNA after digestion with EcoRI (1); Bgl II (2); BamHI (3); Pvu II (4); KpnI (5); HindIII (6) was blot-hybridized with labelled oligonucleotide RII. Identical sizes of the hybridizing fragments on lanes 2 and 4 – 6 let to conclude that putative length of repeating rDNA unit in *Laudakia caucasia* lizards approximates to 10 kb. *b* – *e*: Digestion of lizard genomic DNA with EcoRI (1); EcoRI + SalGI (2); EcoRI + Pst I (3); EcoRI + AccI (4); EcoRI + Bgl II (5); EcoRI + EcoR V (6); EcoRI + NotI (7); EcoRI + Xho I (8) and successive blot-hybridization with RI (*b*), RIII (*c*), RII (*d*), or RIV (*e*). Positions of all these specific oligonucleotide probes are shown on the rDNA restriction map.

3. Alignment of the rDNA regions containing 3′ end of 18S rDNA, ITS-1, 5.8S rDNA, and ITS-2 from *Rattus norvegicus* (Rat), *Lacerta strigata* and *Xenopus laevis* (X.L.) using "DNA Star" program (Clustal). The identical nucleotide positions are shown in dark gray, pairwise homology in gray, and absence of homology in black.

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