Available online at www.sciencedirect.com

SCIENCE DIRECT

Gene 349 (2005) 153–164

GENE
SECTION
EVOLUTIONARY GENOMICS

www.elsevier.com/locate/gene

The first characterisation of the overall variability of repetitive units in a species reveals unexpected features of satellite DNA

Isidoro Feliciello[a], Orfeo Picariello[b], Gianni Chinali[a,*]

[a]*Dipartimento di Medicina Clinica e Sperimentale, Facoltà di Medicina e Chirurgia, Università di Napoli Federico II, Via Pansini 5, I-80131 Napoli, Italy*
[b]*Dipartimento di Zoologia, Facoltà di Scienze MM. FF. NN., Università di Napoli Federico II, Via Mezzocannone 8, I-80134, Napoli, Italy*

Received 5 May 2004; received in revised form 21 October 2004; accepted 6 December 2004
Available online 14 March 2005
Received by M. Di Giulio

Abstract

We investigated the overall variability of the S1a satellite DNA repeats in ten European populations of *Rana temporaria* by a new procedure that determines the average sequence of the repeats in a genome. The average genomic sequences show that only 17% of the S1a repeat sequence (494 bp) is variable. The variable positions contain the same major and minor bases in all or many of the population samples tested, but the percentages of these bases can greatly vary among populations. This indicates the presence in the species of an enormous number of repeats having a different distribution of bases in these variable positions. Individual genomes contain thousands of repeat variants, but these mixtures have very similar characteristics in all populations because they present the same type of restricted and species-specific variability. Southern blots analyses and sequences of cloned S1a repeats fully support this conclusion. The S1 satellite DNA of other European brown frog species also presents properties indicating the same type of variability.

This first characterisation of the overall repeat variability of a satellite DNA in a species has revealed features that cannot be determined by gene conversion and crossing over. Our results suggest that a specific directional process based on rolling circle amplification should play a relevant role in the evolution of satellite DNA.

Keywords: Satellite DNA; Repeat variability; Concerted evolution; Rolling circle; *Rana*

1. Introduction

Highly repetitive DNA sequences organised in long direct tandem arrays are defined as satellite DNA and may constitute a relevant part of the genomic DNA of eukaryotic organisms. The large clusters of satellite DNA sequences are mostly located at centromere and telomeres of chromosomes, and represent a consistent part of the DNA present in the constitutive heterochromatin (Brutlag, 1980; Beridze, 1986).

So far, no defined function has been clearly demonstrated for this highly repetitive DNA, although it could play a role in the genomic structure and evolutionary processes (Bostock, 1980). A possible contribution of centromeric satellite DNA to the centromere function has been also suggested (Henikoff, 2000). Various models have been proposed for the origin and evolution of satellite DNA (e.g. Smith, 1976; Dover, 1982; Charlesworth et al., 1994), but unequivocal experimental evidence in favor of any of these models is lacking. This repetitive DNA shows very variable sequence and size of the repetitive unit among species (Brutlag, 1980), but similar satellite DNAs are frequently present in species of the same species-group or genus (Beridze, 1986).

A fundamental reason for our limited knowledge is that satellite DNA represents the portion of the eukaryotic genomes less accessible to structural analysis. Its organisa-

Abbreviations: bio-16-dUTP, biotin-16-2′-deoxy-uridine-5′-triphosphate; bp, base pair(s); kb, kilobase(s) or 1000 bp; Myr, million(s) of years; PCR, polymerase chain reaction; *R.*, *Rana*; sat-proteins, satellite DNA-binding proteins.

* Corresponding author. Tel.: +39 081 7463355; fax: +39 081 7463367.
E-mail address: chinali@unina.it (G. Chinali).

0378-1119/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2004.12.001

tion in long tandem arrays not only prevents the use of the clone overlapping and chromosome walking techniques needed to assemble long sequences, but also makes the analysis of recombination, amplification and substitution events in this DNA extremely difficult or impossible (Charlesworth et al., 1994).

The S1 satellite DNA of the European brown frogs was firstly characterised in *R. italica* (Cardone et al., 1997), and immediately presented features able to overcome one of the limitations usually met in the study of this DNA. The presence of two homologous repetitive units S1a (494 bp) and S1b (285 bp) in the same satellite DNA made possible to obtain evidence of specific processes that determine very large differences in the organisation of these repetitive units among frogs of the same population. In a more recent study (Picariello et al., 2002), we found that the mixture of the S1a or S1b repetitive units amplified by PCR from the genomic DNA of *R. graeca* could be directly sequenced to yield the average sequence of these two repeats in the genome.

The S1 satellite DNA from *R. temporaria* can be also investigated by this approach. Differently from *R. italica* and *R. graeca*, this brown frog has a very wide geographic distribution, being present in all of the Europe except for the southern parts of the Balkan Peninsula, Italy and Spain. The analysis of the S1 satellite DNA in various populations of *R. temporaria* has also been facilitated by the presence of only one type of repeat (S1a). The possibility to examine different populations living in distant regions separated by geographic barriers that limit or abolish a mutual genetic flow is fundamental to define the dynamics of a satellite DNA within a species.

In this report we have examined DNA samples from ten different European populations of *Rana temporaria* both by Southern blot analysis and by determining the average genomic sequence of the S1a satellite DNA repeat. Additional information has been obtained by the analysis of the sequence of the S1a repeat clones. Our report provides the first complete characterisation of the overall variability of a satellite DNA repeat within a species with a wide geographic distribution. This variability strongly suggests the presence of a specific directional process acting on this repetitive DNA, because it has features that cannot originate only from the action of stochastic processes as postulated by most current models of satellite DNA evolution.

2. Materials and methods

2.1. Animals

We analyzed specimens from ten populations of *R. temporaria* (A–J) of the following areas: Leon, Spain (A); Lourdes, France (B); Bristol, UK (C); Chamberry, France (D); Cuneo, Italy (E); Sondrio, Italy (F); Znojmo, Czech Republic (G); Copenhagen, Denmark (H); Lund, Sweden (I); Ekaterinenburg, Russia (J).

2.2. DNA isolation

DNA was always extracted from blood, skin or liver of single animals. Tissue homogenates were digested at 55 °C with proteinase K (0.2 mg/ml) in the presence of 0.5–1% sodium dodecyl sulphate before extraction with phenol, phenol-chloroform (1:1 v/v) and chloroform-isoamyl alcohol (25:1 v/v). After ethanol precipitation, samples were treated with RNase and proteinase K and processed as previously described (Cardone et al., 1997) to yield highly purified DNA. Results were independent of the tissue used for DNA preparation. DNA from clones of *R. temporaria* S1 satellite DNA in pTZ19R cloning vector (Pharmacia, Uppsala, Sweden) was isolated by a modified alkaline lysis method (Feliciello and Chinali, 1993).

2.3. Molecular cloning

DNA fragments containing whole S1a repeats were obtained by digestion of genomic DNA from *R. temporaria* with *Eco*RV. These fragments were cloned in the *Sma*I site of pTZ19R following a procedure previously described (Cardone et al., 1997).

2.4. PCR amplification

Because of the tandem organisation of satellite DNA, whole unit repeats of S1a from *R. temporaria* are amplified by PCR using primers with the same origin in the S1a sequence, but with opposite orientation. As previously reported, genomic DNA from single frogs was amplified with the two sets of primer pairs Rdr160–Rin161 and Rdr371–Rin372 and the resulting amplified S1a monomers were isolated by preparative gel electrophoresis (Picariello et al., 2002). The two sets of primers were chosen in two regions of the S1a sequence that are highly conserved in brown frogs, and have origins located at a distance of 210 bp in the sequence of the S1a repetitive unit.

2.5. DNA sequencing

After purification, the PCR-amplified S1a repeats were sequenced in both orientations by an automatic sequencer (Applied Biosystems, Foster City, Calif.) using the Big-Dye Terminators kit by the same manufacturer and the primers used for amplification. Each amplification product originates from a large number of similar S1a repetitive units and, as expected, electropherograms showed the presence of more than one base in several positions of the sequence. The direct and inverse sequence electropherograms of each amplified repeat were compared with Sequence Navigator software (Applied Biosystems): only bases present in both sequencing orientations were considered. The portion of sequence around each primer pair was determined by sequencing the amplification