A new *Eco*RI family of satellite DNA in lampreys

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Abstract Satellite DNA sequences have been studied in several groups of organisms. However, until now this type of sequence has not been characterized in cyclostomata, an evolutionarily important class of vertebrates. In the present work, we report the molecular characterization of a new family of satellite DNA in lampreys (*Petromyzon marinus*). Digestion of lamprey DNA with *Eco*RI identified a series of very abundant AT-rich (60% A+T) repeating units, with short stretches of AT, that are multimers of 370 bp. Southern blot analysis and comparison with the satellite DNA sequences deposited in the databases indicate that this new family of satellite DNA is exclusive to lampreys. The distribution of this *Eco*RI satellite DNA on lamprey chromosomes was analyzed by in situ hybridization. The evolutionary origin of this satellite is briefly discussed.

Key words: Lamprey; Satellite DNA; Chromosome in situ hybridization

1. Introduction

The eukaryotic genome contains multiple copies of related DNA elements that form families of repetitive sequences that may be reiterated 10–10^5 per haploid genome and may constitute as much as 66% of the genome [1–3]. Satellite DNA is a kind of these sequences characterized by very highly repetitive and tandemly repeated units comprising large clusters (up to 100 megabases) that contribute to the bulk of DNA in heterochromatic regions [4,5]. The origin and evolution of satellite DNA remains unclear and several mechanisms such as saltatory replication or unequal crossing-over have been proposed [5].

No defined function has yet been assigned to satellite DNA although it could have a role in genome structure and evolutionary processes [6]. A structural function of centromere-associated satellite DNA has been proposed that confers centromere functionality [7–9]. In some species, common characteristics of these sequences are a high AT content with short stretches of AT [10,11] and the presence of sequence motifs that may be involved in protein binding.

Satellite DNA sequences have been studied in several groups of organisms including mammals [1,12–14], amphibians [15], teleost fishes [10,16,17] and invertebrates [18–20]. However, no information is available about the sequence, organization and chromosomal distribution of this type of DNA sequences in cyclostomata, an evolutionarily important class of vertebrates. In this report, we describe the isolation, characterization and cytogenetic location of an *Eco*RI satellite DNA from *Petromyzon marinus*, a well-known member of this group of vertebrates.

2. Material and methods

2.1. DNA cloning and sequencing

Lamprey DNA was prepared from muscle following standard procedures [21]. DNA (10 μg) were digested overnight with *Eco*RI (16 U/μg) and electrophoresed through a 1% low-melting agarose gel. Staining with ethidium bromide showed a visible ladder starting in a band of about 370 bp. This first (~370 bp) and the second (~740 bp) bands of the ladder were excised from the gel, purified and ligated to the *Eco*RI site of pGem3Z (Promega). The recombinant plasmids were named pBL6 for the 370 bp insert (monomeric unit) and pBL2.7 for the 740 bp insert (dimer). The nucleotide sequences of the pBL6 and pBL2.7 inserts were determined using the chain-termination method [22] and the Sequenase system (Amersham).

2.2. Southern blot analysis

For Southern blot hybridization, DNAs were digested with 16 U of *Eco*RI per μg of DNA, fractionated in 1% agarose gel, transferred to Hybond N^+ membranes (Amersham) and hybridized with the 370 bp unit labelled by PCR. For the PCR labelling reaction, the 370 bp unit was amplified from the pBL6 using 1 ng of DNA, 50 μCi of [α-^{32}P]dCTP (3000 Ci/mmol, Amersham), 200 μM of the other dNTPs, 0.2 μM of the M13 forward and reverse universal primers, 1×*Taq* buffer (BRL), 1.5 μM of $MgCl_2$ and *Taq* DNA polymerase (5 U) per 100 μl of reaction. The cycles of amplification were as follows: 29 cycles of 1 min at 94°C for denaturation, 50 s at 50°C for annealing and 1 min at 72°C for extension, with 5 min extension at 72°C after the final cycle. The specific activity of the probe was 2×10^8 cpm/μg. Hybridization was carried out overnight at 42°C in 10 ml of a solution containing 5×SSC (1× = 0.15 M NaCl, 15 mM trisodium citrate pH 7.0), 5×Denhart's solution (1× = 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll), 50% formamide, 10% dextran sulfate, 100 μg/μl of denatured salmon sperm DNA and 4×10^6 cpm/ml of the denatured probe. The membranes were washed for 15 min at RT in 2×SSC and 0.5% SDS, twice for 30 min at 65°C in 1×SSC and 0.5% SDS and twice for 30 min at 42°C in 0.1×SSC and 0.5% SDS, followed by autoradiography at −70°C with intensifying screens.

2.3. Chromosome in situ hybridization

Mitotic chromosomes were obtained from spontaneously dividing cells of kidney and peripheral blood [23] and from lymphocyte cultures obtained starting from blood [24] or kidney [25]. For in situ hybridization, mitotic chromosomes were treated with RNase (100 μg/ml) in 2×SSC, dehydrated in an ethanol series (70%, 95% and 100%) for 5 min and air-dried. Denaturation was carried out using formamide (70% in 2×SSC for 2 min at 70°C), followed by dehydration in an ethanol series again and air-drying. Preparations were incubated in a moist chamber overnight at 37°C with 50 μl hybridization solution (50% formamide, 2×SSC, 10% dextran sulfate, 500 ng/ml sonicated herring sperm and 100 ng of biotinylated probe). The probe (the monomeric unit cloned in pBL6) was labelled with biotin 16-dUTP by nick translation (Boehringer Mannheim). The slides were washed for 10 min in 50% formamide and 2×SSC at 42°C and then twice for 5 min at 37°C in 2×SSC. Hybridization was detected with fluorescein-conjugated avidin (Vector Laboratories). The signal was enhanced by incubation with biotinylated goat anti-avidin (Vector)

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We would like to dedicate this work to the University of Santiago on the occasion of the fifth centenary of its foundation.

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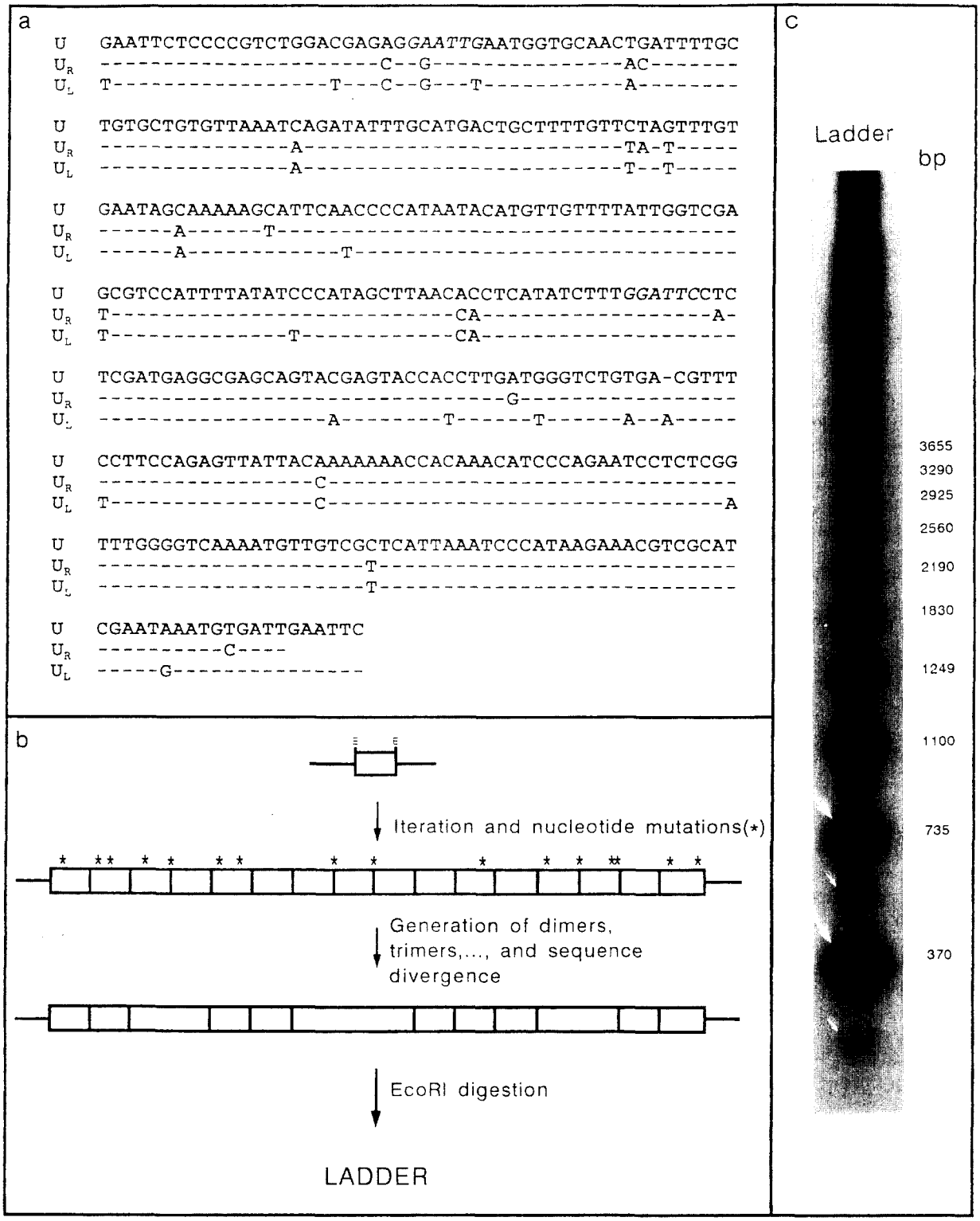

Fig. 1. Molecular characterization of a lamprey satellite DNA. a: Comparison of the nucleotide sequences using the Clustall program. Only base changes between sequences are shown. U represents the 370 bp repeat unit; U_L and U_R correspond to the left and right units of the dimer. The sequence that could be recognized by the star activity is in italics. b: Hypothetical model explaining the generation of the satellite. E, *Eco*RI; boxes represent the monomer, dimer, trimer. c: Southern blot analysis of lamprey DNA digested overnight with *Eco*RI and hybridized with the 370 bp *Eco*RI fragment purified from agarose gels and cloned into pGEM3z. Big arrows mark the monomer and the dimer and small arrows mark the fragments likely originated by the endonuclease star activity.

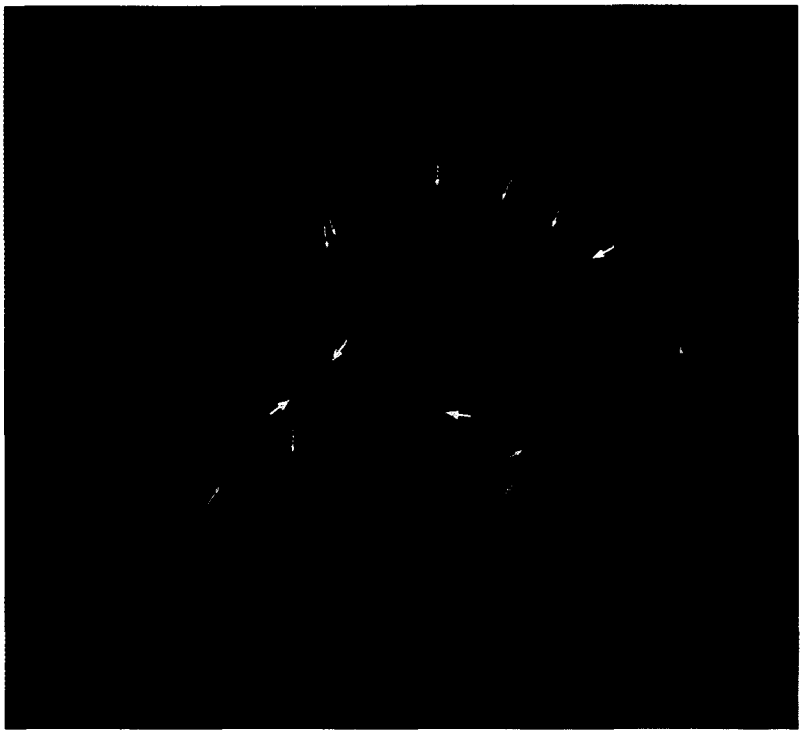

Fig. 2. Metaphase chromosomes from *Petromyzon marinus* following fluorescent in situ hybridization with *Eco*RI satellite. Big arrows indicate the hybridization signal at centromeric and paracentromeric areas in two pairs of two-armed chromosomes. Small arrows show the hybridization at centromeric regions in some acrocentric chromosome pairs.

followed by fluoresceinated avidin. The chromosomes were counterstained with propidium iodide (1 μg/ml in PBS for 1–5 min).

3. Results and discussion

When *Petromyzon marinus* DNA was digested with *Eco*RI and fractionated in agarose gel, we observed a group of bands, multimers of ~370 bp with a ladder structure. For the molecular characterization of this ladder, the minor band of ~370 bp (monomeric unit) and the ~740 bp band (dimer) were isolated from a low-melting agarose gel and cloned in the pGem3Z plasmid obtaining the recombinant clones pBL6 (the insert is the 370 bp fragment) and pBL2.7 (the insert is the 740 bp fragment). Fig. 1 shows the sequence of the 370 bp unit (U). Sequence analysis of the dimeric fragment demonstrated that it is formed by two copies in tandem (U_L-U_R) of the 370 bp unit indicating that the structure of the other ladder bands is also in tandem. Comparison among these sequences revealed a low degree of divergence (Fig. 1). The percentages of similarity are: U-U_L, 93.27% (25 mutations); U-U_R, 95.07% (18 mutations); U_L-U_R, 94.80% (19 mutations). Computer search at the GenBank/EMBL databases did not find any homology with known DNA sequences. Both DNA sequences have been deposited in the EMBL database with the accession numbers X92515 (monomer) and X92516 (dimer).

To have an estimate of the abundance and the size of this satellite, we carried out a Southern blot analysis of lamprey DNA digested with *Eco*RI. After fractionation on 1% agarose gel and subsequent blotting, the filter was hybridized with the 370 bp unit labelled with PCR. As shown in Fig. 1, digestion of lamprey DNA with *Eco*RI identifies a series of very abundant repeating units that are multimers of 370 bp and also a minor series of half repeats. In contrast to what was found in other related vertebrates [10,16,17], the ladder obtained after *Eco*RI digestion was not originated by partial digestion because adding more enzyme or longer incubation times did not vary its structure (data not shown). On the other hand, the minor ladder observed in Fig. 1 could be originated either by an *Eco*RI star activity or by mutations in the *Eco*RI-like sites (indicated by italics in Fig. 1) present in some tandem elements of the satellite or both. Digestion of pBL6 and pBL2.7 with *Eco*RI also generated the minor bands indicating that at least some of these bands are originated by a star activity.

To determine if this new *Eco*RI satellite found in lamprey is present in phylogenetically related or unrelated species, Southern zooblot analysis of *Eco*RI-digested DNAs from different mammals (primates, murine and rodents), birds (chicken), reptiles (*Lacerta sp.*), amphibians (*Rana sp.*), fishes (*Raja sp.*, *Salmo trutta*), cephalochordata (*Amphioxus lanceolatus*), echinodermata (*Paracentrotus sp.*), mollusca (*Venerupis sp.*) and insecta (*Drosophila melanogaster*), was carried out under the same conditions employed above. We observed no hybridization with the 370 bp probe (data not shown) suggesting that this satellite is present only in lampreys.

In situ hybridization of the biotinylated monomeric unit to denatured metaphase spread revealed specific labelling at centromeric regions in some acrocentric pairs and centromeric and paracentromeric areas in two pairs of two-armed chromosomes (Fig. 2). The sequence analysis of this satellite DNA showed a high AT content (60%) common to other centromeric satellite DNAs described in mammals [26], newts [27] and fishes [10,11]. Moreover, we have found the presence of short repeats such as CTG(A/C)A(A/T), GA(C/T/G)AAAAC

or (C/G)AAAA(C/G) that are very similar to other centromeric motifs described in other vertebrates [11]. These sequence motifs could be important elements in centromere structure and function [11,28,29].

A possible explanation for the evolution of the hierarchical variations observed in this satellite is the mechanism known as saltatory replication [2,30,31]. According to this model, the 370 bp repeated unit was amplified laterally to generate many identical tandem copies. Then the copies diverged in sequence as mutations accumulated in them (Fig. 1). If some of the mutations took place in the *Eco*RI site, they would originate the ladder structure. Since the divergence among the copies amplified is low, the period of time that has passed from the last replicatory mechanism should be short unless some selective pressure were taking place.

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