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Sequence divergence and conservation in organizationally distinct subfamilies of *Donax trunculus* satellite DNA

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Abstract

Characterization of a low-copy number DTF1 satellite DNA detected in the bivalve mollusk *Donax trunculus* revealed extensive grouping of monomer sequence variants into subfamilies identified by distinctive combinations of diagnostic nucleotides. It can be anticipated that a large number of subfamilies exists in the genome. In addition to the tandem organization of 169 bp long monomers, at least one subfamily was created through amplification of adjacent repeats in a higher order register. This complex satellite unit consists of two distinctive monomer variants that differ both in specific nucleotide changes and in a deleted segment partially substituted with a short unrelated sequence element. Most of the nucleotide substitutions differing between subfamilies are highly homogenized within a corresponding group of monomer variants, and intra-subfamily variability in general is low. Nucleotide diversity analysis of all sequenced variants of DTF1 satellite revealed the presence of two conserved segments, while the rest of the monomer sequence shows uniform and considerably higher level of variability. The persistence of conserved segments stands in contrast to the sequence and organizational divergence of monomer variant groups, and may indicate constraints in the evolution of DTF1 satellite repeats.
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1. Introduction

Satellite DNAs are highly reiterated, tandemly repeated, non-coding sequences which are also major constituents of heterochromatic genomic compartments. They evolve rapidly and often differ in copy number, nucleotide sequence and composition of repeats among even closely related taxa, thus making satellite profiles species-specific (Ugarković and Plohl, 2002). Repetitive sequences evolve in concert through a stochastic process of molecular drive during which mutations are homogenized within a genome and fixed in a reproductive population (Dover, 1986, 2002). Differences in rates of nonreciprocal DNA exchanges within and between chromosomes stimulate sequence homogenization within a subset of satellite monomers, and formation of distinctive groups or subfamilies of repeat variants (Dover, 1986; Schindelhauer and Schwartz, 2002). On the other hand, similar intragenomic rates of DNA exchange have been invoked to explain both the lack of subfamilies and random arrangement of satellite repeat variants (Plohl et al., 1992).

Several, or even many, satellite DNA families usually coexist in a genome, differing significantly in copy number due to random amplification of repeat elements (Ugarković and Plohl, 2002; Pons et al., 2004). Among mollusks, three satellite DNAs coexisting in genomes of four *Mytilus* species were comparatively analysed and justified as markers in phylogenetic analysis (Martínez-Lage et al., 2002). Grouping of satellite monomers into distinctive subfamilies was anticipated for the satellite DNA from Antarctic scallop *Adamussium colbecki* (Canapa et al., 2000). The most diverse molluskan system consisting of five satellite DNAs has been characterized in the clam *Donax trunculus*. Besides similarities in monomer length of about 160 bp, four of the satellites share *Hin*dIII sites and a complex monomer sequence built of 6 to 15 times repeated

Abbreviations: bp, base pairs; NJ, Neighbour Joining method; HKY+G, Hasegawa–Kishino–Yano 1985 model with gamma distribution; S.D., standard deviation; hLRT, hierarchical likelihood ratio test; HOR(s), higher-order repeat(s).

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oligonucleotide motifs GGTCA and GGGTTA (Plohl and Cornudella, 1997). Tandem arrays of the latter motif have also been located in terminal telomeric positions of all chromosomes in mollusks *D. trunculus* and *Mytilus galloprovincialis* (Plohl et al., 2002). Monomer variants of another, *Eco*RV, family lack any internal substructure but they can be divided into two subfamilies characterized by highly homogenized diagnostic nucleotides (Plohl and Cornudella, 1996). This satellite appears to be related to a MITE-like transposable element *pearl* found in the genome of the American oyster *Crassostrea virginica* (Gaffney et al., 2003). The similarity of this element with other related satellites detected in various oyster species indicates that it could be a member of a satellite family widely distributed in mollusks (López-Flores et al., 2004).

However, information concerning both possible origins of diverse satellite sequences within a genome and constraints on their evolution is scarce in general and particularly in mollusks. Here, the characterization of a novel satellite DNA family from *D. trunculus* revealed the presence of homogeneous subfamilies distinct in both nucleotide sequence and organization of repeat units. In addition, the conservation of segments of the satellite repeat throughout subfamilies indicates evolutionary constraints and putative functionality of this repetitive sequence.

2. Materials and methods

2.1. Cloning and sequencing of satellite repeats

Genomic DNA was extracted from adult specimens of *D. trunculus* obtained from commercial suppliers, during the stay of one of us (MP) in the laboratory of Dr. Luis Cornudella at the Institute of Molecular Biology of Barcelona (IBMB-CSIC, Spain). The standard phenol protocol was used to obtain DNA from fresh suspensions of sperm cells as described previously (Plohl and Cornudella, 1997). Genomic DNA was digested with appropriate restriction endonucleases according to the manufacturer's instructions and fragments separated by electrophoresis on 1% agarose gels. After electrophoretic separation, DNA material was purified from bands of interest with the Qiaquick Gel Extraction Kit (Qiagen) and ligated into the pUC18 plasmid vector. Aliquots of eluted fragments were labelled with digoxigenin by random priming method, using the DIG DNA Labelling and Detection Kit (Roche) and used as hybridization probes for screening of recombinant colonies. The cloned fragments were sequenced by MWG-Biotech sequencing service (Ebensberg, Germany). The cloning efficiency of monomeric repeats belonging to this satellite family was extremely low, and positives had to be collected in several cycles. All attempts to clone any of the satellite DNA multimers remained unsuccessful.

2.2. Southern and dot-blot hybridization experiments

Genomic DNA samples (5 μg) were digested with restriction enzymes, electrophoresed on 1% agarose gels and transferred onto positively charged nylon membrane (Roche). Membranes were hybridized in 20 mM sodium phosphate buffer (pH 7.2), 20% SDS. The exact temperatures are listed later in descriptions of particular experiments. Stringency washing was performed in 20 mM Na_2HPO_4, 1% SDS at the temperature three degrees lower than the hybridization temperature. The relative genomic contribution of the satellite DNA was determined in dot-blot analysis. Serial dilutions of genomic DNA and cloned DTF1 variants were spotted onto a nylon membrane. In order to detect all members of the family, hybridization was performed with a mix of labelled representatives of DTF1 monomer variants under relatively relaxed stringency conditions (65 °C).

2.3. Sequence analysis

Nucleotide sequences were aligned using the default parameters of the ClustalX v. 1.81 (Thompson et al., 1997). Sequence divergences were calculated according to the best-fit model of nucleotide evolution HKY85+G (Hasegawa-Kishino-Yano with gamma distribution; Hasegawa et al., 1985) selected by the hierarchical likelihood ratio test (hLRT) in Modeltest 3.06 (Posada and Crandall, 1998). Distance trees were built by the Neighbour Joining (NJ) method implemented in PAUP* v. 4b10 computer package (Swofford, 1998). Bootstrap values were calculated based on 1000 replicates.

The distribution of nucleotide differences and DNA polymorphisms was analysed using DnaSP v.3.99 program (Rozas and Rozas, 1999) as described previously (Mravinac et al., 2004). Conserved and variable segments in satellite DNA sequences were determined by sliding window analysis using window size of 10 bp and step size 1. The segments with nucleotide diversity values deviating for more than 2 S.D. from the average were considered variable.

3. Results

3.1. Cloning and sequencing of HinfI repeats

Digestion of *D. trunculus* genomic DNA with *Hin*fI restriction endonuclease generates a regular ladder of electrophoretic bands which indicates the presence of tandemly arranged repetitive sequences. Cloning and sequencing of DNA material extracted from the monomeric band revealed two types of sequences we named DTF1 and DTF2 (for *D. trunculus Hin*fI). Even though both satellites have the 169 bp long repeating unit, they differ in all other aspects. For instance, the average A+T content of the DTF1 satellite equals 61.7%, while the DTF2 satellite is A+T poor (37.5%). Since the two groups of monomers are unrelated both in DNA sequence and genomic organization, only repeats belonging to the DTF1 family are analysed here, and the characterization of DTF2 satellite will be reported elsewhere. Genomic abundance of the DTF1 satellite family was determined by dot-blot experiments (not shown). DTF1 builds only 0.1% of *D. trunculus* genome, which is equivalent to 8200 copies according to the haploid genome size of 1.4×10^9 bp as determined by Hinegardner (1974).

Sequence alignment of 10 randomly cloned monomers revealed that the DTF1 satellite family can be divided into two subfamilies (DTF1A and B) on the basis of characteristic

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