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PREGLEDNI ČLANCI **REVIEWS**

THE SPECIES-SPECIFICITY AND EVOLUTION OF SATELLITE DNAs WITH EMPHASIS ON SATELLITE DNAs IN TENEBRIONID BEETLES

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Satellite DNAs are highly reiterated non-coding sequences, organized in long arrays of tandem repeats that represent major DNA constituents of heterochromatic genome compartments. Satellite monomers build domains that stretch over functionally important regions such as that of the functional centromere. In short evolutionary periods satellite DNA components often change rapidly in copy number, nucleotide sequence, or both. Species specific profiles of satellite repeats can be formed by differential amplification of sequences coexisting in the genomes of a group of related organisms as a common library of satellite DNAs. This hypothesis has been experimentally approved for the first time in an analysis of satellite sequences in species of the genus Palorus (Insecta, Coleoptera). Due to the complexity of their evolution, the application of satellite DNAs in phylogenetic studies has limitations and results should be interpreted cautiously. Nevertheless, satellites can help to resolve phylogenetic relationships in some cases and in addition they can represent excellent and reliable molecular markers in the identification of some organisms.

Heterochromatin, repetitive sequences, concerted evolution, library hypothesis, molecular markers, phylogenetic studies, *Tenebrio molitor*

PLOHL, M. Vrsna specifičnost i evolucija satelitnih DNA s naglaskom na satelitne DNA kornjaša iz roda *Tenebrio*, Zavod za molekularnu biologiju, Institut Ruđer Bošković, Bijenička 54, HR-10002 Zagreb, Hrvatska, plohl@irb.hr - Entomol. Croat. 2005, Vol. 9. Num.1-2: 85 - 96

Satelitne DNA su visokoponovljene nekodirajuće sekvence organizirane kao dugi nizovi uzastopnih ponavljanja koji predstavljaju glavne elemente DNA u heterokromatinskim genomskim odjeljcima. Domene satelitnih monomera protežu se i u funkcionalno značajnim područjima kao što su centromere. Satelitne se DNA najčešće već tijekom kratkih evolucijskih razdoblja brzo mijenjaju u broju kopija, nukleotidnoj sekvenci ili i u jednom i u drugom. Vrsno specifični profili satelitnih jedinica ponavljanja mogu

nastati diferencijalnom amplifikacijom sekvenci koje istovremeno postoje u genomima skupine srodnih organizama kao zajednička biblioteka satelitnih DNA. Ova pretpostavka je prvi puta bila eksperimentalno dokazana tijekom analize satelitnih sekvenci u vrstama roda *Palorus* (Insecta, Coleoptera). Zbog složenosti njihove evolucije, primjena satelitnih DNA u filogenetskim studijama ima ograničenja i rezultati se moraju interpretirati oprezno. Usprkos tomu satelitne DNA mogu u nekim slučajevima pomoći u razrješavanju filogenetskih odnosa te također predstavljati izvrsne i pouzdane molekularne biljege u identifikaciji pojedinih organizama.

Heterokromatin, ponovljene sekvence, usklađena evolucija, hipoteza biblioteke, molekularni biljezi, filogenetske studije, *Tenebrio molitor*

Heterochromatic genome compartment

Heterochromatin is a ubiquitous component of animal and plant genomes, located mostly in pericentromeric and telomeric chromosomal regions. According to its cytological definition, those regions in eukaryotic chromosomes that remain condensed throughout the cell cycle are heterochromatic (Heitz, 1928). Heterochromatic regions comprise a considerable fraction of eukaryotic genomes, for example about 30% of the human and Drosophila genomes (John and Miklos, 1979) or 50% of the mealworm beetle *Tenebrio molitor* (Weith, 1985). The dominant DNA components in heterochromatin are highly reiterated DNA sequences historically named satellite DNAs because of their buoyant density, which is different from the rest of genomic DNA in experiments with gradient centrifugation (Szybalski, 1968). Basic repeating units or satellite monomers build megabase-sized arrays that are a hallmark of heterochromatin. The apparent lack of transcription and of any gene-coding function led to the conclusion that satellite DNAs are just DNA waste discarded into a genetically inert heterochromatic compartment in which they accumulate due to favorable sequence dynamics in regions around centromeres and at telomeres (Ohno, 1972; Stephan and Cho, 1994). The misty atmosphere around heterochromatin is enhanced by the strange effects heterochromatin exerts in addition to being permanently condensed, such as a low rate or lack of standard meiotic recombination, replication of DNA in the late S phase and the silencing of euchromatic genes. For all these reasons, heterochromatin and satellite DNAs were for a long time left out of the mainstream of research interests. In addition, the difficulties in cloning and the inability of current techniques to assemble shotgun DNA segments composed of repetitive elements caused heterochromatic compartments to remain poorly

represented in outputs of genome projects (Henikoff, 2002; Nagaki et al., 2004; Rudd and Willard, 2004).

Despite numerous difficulties, satellite DNAs and heterochromatin started to attract considerable attention thanks to recent results favoring their fundamental importance in the function and evolution of eukaryotic genomes. For example, long homogeneous domains of satellite repeats, occasionally interrupted with transposable elements, turned out to be the principal DNA components involved in centromeric function of animal and plant species, such as Drosophila (Sun et al., 2003), human (Schueler et al., 2001), and rice (Cheng et al., 2002). Recent results indicate that many satellite DNAs are transcribed and that transcripts of satellite repeats in the form of small interfering RNAs are involved in the initiation of histone H3 methylation, a necessary prerequisite in heterochromatin formation and maintenance (Aravin et al., 2003; Martienssen, 2003). The phenomenon of variegated silencing of euchromatic genes transferred in the vicinity of heterochromatin by chromosomal rearrangements, known as position-effect variegation (PEV), can lead to a mosaic pattern of expression of the affected gene. A number of modifier genes were identified according to mutations that suppress or enhance PEV, and many of these genes encode non-histone heterochromatin proteins (Schotta et al., 2003). Nevertheless, the most intriguing is the finding of transcriptionally active essential genes in the repressive environment of heterochromatin. These genes are embedded into arrays of satellite sequences and require the heterochromatic environment for their normal activity; if translocated to euchromatin their expression is impaired (Howe et al. 1995). Until now, more than 20 genes have been identified, 16 of them being essential, in the pericentromeric heterochromatin of chromosome 3 in *Drosophila melanogaster* (Fitzpatrick et al. 2005). However, it is not known if comparable heterochromatic genes exist in other organisms. Sequence analysis of over 400 kb of pericentromeric DNA in the human X chromosome, as well as pulsed-field analysis of the whole region, did not reveal any other sequences in addition to satellite DNAs and transposable elements (Schueler et al., 2001).

Satellite DNAs in tenebrionid beetles

Satellite DNAs can be easily detected after digestion of genomic DNA with restriction endonucleases capable of cutting within a satellite sequence. If the selected restriction endonuclease cuts only once within a monomer, partial dige-

Fig. 1. Electrophoretic separation of fragments in time-course digestion of genomic DNA. If restriction endonuclease cuts once within the satellite monomer, a ladder of DNA fragments is visible, corresponding to multimers of a satellite monomer. The ladder is shorter with a progress in time from partial to total digestion. Due to sequence variability, a fraction of restriction sites is inaccessible to the endonuclease and some multimeric fragments persist even in conditions of total restriction endonuclease digestion.

stion and subsequent electrophoretic separation of DNA fragments will produce a characteristic ladder pattern of monomer multimers. The ladder is longer at short time-intervals of digestion and collapses to fragments corresponding to the monomer length after prolonged, complete digestion (Fig. 1). This simple approach led to the detection, cloning and characterization of satellite DNAs in many animal and plant species.

Beetles of the family Tenebrionidae (Insecta, Coleoptera) are typically characterized by highly abundant satellite sequences comprising up to 50 % of genomic DNA and located in massive blocks of pericentromeric heterochromatin

of all chromosomes (for example in beetles of the genus *Palorus*; Meštrović et al., 2000). In that sense tenebrionid beetles represent a suitable model system for the characterization of satellite DNA sequences, their organization and evolution in pericentromeric heterochromatin (Petitpierre et al., 1995). Sequence variability of repeating units in tenebrionid satellites is low, usually only 2-3% mostly due to nucleotide substitutions. The monomer length of these satellites falls into two groups, between 100-160 bp and about 340 bp (Ugarković et al., 1995), similar to that observed in many other satellites (John and Miklos, 1979). One or two satellite DNAs can be easily detected in each tenebrionid species as highly abundant or major satellite components. These satellites usually represent unrelated entities and no mutual relationship or ancestral sequence could be discerned in sequence comparisons even among the most closely related species. Instead, some satellites share certain structural characteristics. As an example, highly abundant satellites detected in species of the genus *Palorus* have in common a sequence-induced bent DNA helix axis potentially able to form a structure that might in turn act as a signal in interactions with some protein components (Plohl et al. 1998). Recent results on the structural and organizational properties of satellite sequences in (peri)centromeric heterochromatin and putative functional sequence elements in them have been reviewed in detail elsewhere (Plohl et al., 2004). Unrelated highly abundant satellites such as those observed in congeneric *Palorus species* can misleadingly suggest the evolution of satellite sequences through rapid accumulation of mutations up to an extent at which similarities among them become insignificant.

Satellite DNAs can be highly conserved in nucleotide sequence

The example described above poses a major question about satellite DNAs: how can satellite DNAs accumulate divergences so rapidly even among the most closely related species. Evolution of satellite sequences is governed by principles of concerted evolution, in which mutations are homogenized throughout members of a repetitive family and fixed within a group of reproductively linked organisms in a stochastic process of molecular drive (Dover, 1986). Diverse mechanisms of nonreciprocal transfer such as gene conversion and unequal crossing-over cause sequence homogenization, while fixation results from random chromosome assortment in sexual reproduction. These mechanisms induce high turnover of satellite sequences and rapid changes in copy number, nucleotide sequence and

composition of satellites in the genome, resulting in high within-species homogenity of satellite repeats and alterations in satellite profiles between species.

Some satellite sequences indeed change rapidly in evolution and accumulate mutations even at the population level, for example in the pupfish (Elder and Turner, 1994). On the other hand, some are widely distributed across species, providing evidence of nucleotide sequence conservation for long-term evolutionary periods. A study of Pimelia (also of the family Tenebrionidae) suggested that a satellite DNA, highly abundant in all examined species, has persisted for more than 8 million years (Pons et al., 2002). Satellites in species from the *Drosophila virilis* group remained conserved for about 20 Myr (Heikkinen et al. 1995), and cetacean satellite DNA persisted for at least 40 Myr (Arnason et al. 1992). Low-copy repeats indistinguishable in their nucleotide sequence from high-copy *Palorus ratzeburgii* satellite DNA have been detected in distant species *Pimelia elevata*, although these taxa were separated for 60 Myr (Mravinac et al., 2002). Conservation or "freezing" of repeat families for long evolutionary periods can be explained as a consequence of mechanisms involved in concerted evolution, if a small bias in turnover mechanisms is anticipated (Dover and Flaveli, 1984). Such preference to maintain some putative "optimal" set of monomer variants may be due to functional constraints imposed on the nucleotide sequence (Mravinac et al., 2005). Slow rates of sequence change and of concerted evolution in some satellites were explained as specificity of slow general genomic evolution in sturgeons (Robles et al., 2004) and in whales (Arnason et al., 1992).

The library hypothesis

Several or even many satellite DNAs often coexist in a genome in different copy numbers as a result of independent differential amplification of each of them, as well documented both in plants (King et al., 1995) and in animals (Nijman and Lenstra, 2001). Based on the analysis of distribution of a satellite among the three major suborders of rodents, a hypothesis was raised according to which related species share a collection, or a library of satellite repeats (Salser et al., 1976). According to this model, one or a few of these sequences can be amplified to easily detected high-copy number repeats in one species, while in others these sequences can remain underrepresented and hidden as low-copy repeats. Differential amplification of satellites might therefore be sufficient to explain the species-specificity of satellite DNA profiles and the differences between

highly abundant satellite sequences in closely related species. This hypothesis remained unproved until recently due to the complexity of satellite profiles and the technical difficulties in detection and analysis of low-copy repeats. Using a sensitive PCR technique, analysis of the distribution of four satellites in Palorus provided the first experimentally verified clue to the question how rapid changes of satellite DNAs in heterochromatin could be achieved (Meštrović et al., 1998). The distribution of *Palorus satellites* is as predicted by the library hypothesis: in each tested species one of them is amplified into a high-copy repeat forming 20-40% of genomic DNA, while the others are in the form of low-copy repeats that comprise less than 0.05%. Dramatic alterations in copy number made the satellite profile species specific, while the nucleotide sequence of each satellite could not be discerned according to the species of origin. Phylogenetic analysis based on comparisons of mitochondrial cytochrom oxidase I (COI) gene revealed that the closest among the studied species, *Palorus genalis* and *P. ratzeburgii*, separated at least 7 Myr ago (Meštrović et al., 2000). Differences in satellite profiles of many related taxa can be explained in terms of the library hypothesis such as in the genus *Pimelia* (Bruvo et al., 2003), in the parasitic wasp *Trichogramma brassicae* and its congeneric species (Landais et al., 2000), and in the stick insects *Bacillus rossius* (Cesari et al., 2003). Although in all of the above examples satellite sequences remained conserved, the contribution of mutations and/or sequence rearrangements that may ultimately generate a novel satellite repeat is significant in many systems (Pons et al., 2004, Ugarković and Plohl, 2002). It is not known if the library concept is universal, but the evolution of at least some satellite profiles is probably determined by different combinations of changes in copy number, nucleotide sequence and generation or elimination of repeats (Fig. 2).

Satellite sequences in phylogenetic and taxonomic studies

It has been shown that mutations in satellite repeats accumulate gradually, in accordance with phylogenetic relationships (Bachmann and Sperlich, 1993). As exemplified above, rates of accumulation of changes in a nucleotide sequence differ significantly among satellite DNAs. Depending on the rate of accumulation of mutations able to resolve satellite monomers according to the organism of origin, gradual accumulation of sequence changes follows phylogeny at different hierarchical levels. At the species level, satellite DNAs were informative in phylogenetic studies of the fish family Sparidae (Garrido-Ramos et al., 1999), in

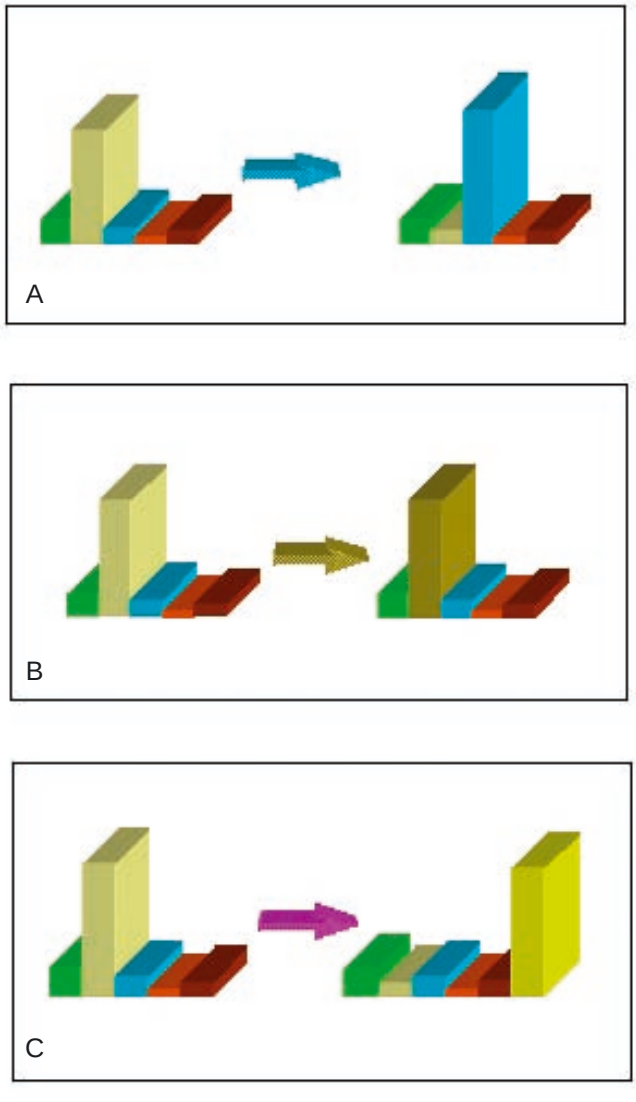

Fig. 2. Satellite DNA profiles can change because of changes in copy number (A), changes in nucleotide sequence (B), or due to generation and/or elimination of satellite repeats (C). Each bar represents one satellite DNA, its height being proportional to the copy number while colors indicate differences in nucleotide sequences.

species from the *Drosophila obscura* group (Bachmann and Sperlich, 1993) or in whales (Arnason et al., 1992). Gradually evolving satellite DNAs were able to resolve ecologically differentiated closely related endemic species of Hawaiian spiders better than mitochondrial DNA markers (Pons and Gillespie, 2004). Since satellite DNAs are inherited from both parents they also overcome limitations of mitochondrial markers due to their maternal inheritance. It has been concluded that "satellite DNA sequences may potentially be very useful for resolving relationships between rapidly evolving taxa within an adaptive radiation" (Pons and Gillespie, 2004). At higher phylogenetic levels, mutations in satellite sequences can resolve geographic species groups in the genus *Pimelia* (Pons et al., 2002, Bruvo et al., 2003). Slow rates of gradual sequence changes can be detected even in some extremely conserved satellites, resolving about 90 Myr old phylogeographic clades of sturgeon fish (Robles et al. 2004).

Major satellite sequences can often be used as a molecular marker which can be extremely useful in the identification of particular taxa. Highly abundant satellites of many organisms are clearly species-specific (Ugarković and Plohl, 2002), as exemplified in the genus *Palorus* (Meštrović et al., 2000). In that sense, satellite DNAs have been used as molecular diagnostic probes for rapid identification of morphologically highly similar individuals of root-knot nematodes of the genus *Meloidogyne* (Randig et al., 2002). As a conclusion, due to the complexity of evolution of satellite profiles, these sequences should be taken cautiously in phylogenetic and taxonomic studies, although they can often represent reliable molecular markers. The major limitations are in rapid and nonuniform evolution of satellite DNA profiles mostly due to random differential amplification of satellite sequences from a library, as well as in large discrepancies in rates of nucleotide sequence evolution of particular satellites. These features can blur sequence comparisons particularly if only highly abundant sequences are considered in the analysis.

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