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ISSR-PCR AND MOBILE GENETIC ELEMENTS IN GENOMES OF FARM MAMMALIAN SPECIES

V.I. Glazko[1], E.A. Gladyr'[2], A.V. Feofilov[1], N.V. Bardukov[1], T.T. Glazko[1]

[1] Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Moscow 127550, Russia

e-mail: vglazko@yahoo.com

[2] All-Russia Research and Development Institute for Livestock Husbandry, RAAS, Dubrovitsy settlement 142132, Moscow province, Russia

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S u m m a r y

Highly polymorphic ISSR-PCR markers, the DNA fragments flanked by invert microsatellite repeats, are widely applied with the aim of the genomic scan (polyloci genotyping). The sequencing of such DNA fragments from domestic horse and cattle genomes testified the close connection between the inverted microsatellite repeats and products of recombination between endogenous retroviruses. Mutual genomic positioning of microsatellites and endogenous retroviruses as a source of polymorphisms of ISSR-PCR markers was discussed.

Keywords: microsatellites, retrotransposons, endogenous retroviruses, ISSR-PCR markers.

Current transition from genetics to genomics, from the study of individual genetic elements to the comparative analysis of entire genomes in nanometric scale opens up new possibilities for investigating genetic structures and their dynamics in different groups of living organisms. Genomic scanning is becoming of particular importance as a basis for the analysis of gene pools of agricultural species, since only using the sufficient amount of genomic molecular markers is the way to preserve, manage, and improve biodiversity of such species. Scanning may be performed at the level of a few tens or hundreds of markers, as well as complete sequencing of a whole genome (1). Today, there are many methods of genome scanning, one of which is in polylocus genotyping for DNA fragments of different lengths flanked by inverted repeats of microsatellite loci (inter-simple sequence repeats - ISSR-PCR markers) (2). Inverted repeats are of particular interest, because the capability of DNA to formation of a secondary structure (bulges, loops) is the basis of genomic instability in points of their location (3).

One of the features of ISSR-PCR markers, as well as other repeats such as SINE, is their uneven distribution over the genome (3, 4). Moreover, as it was found earlier, certain ISSR-PCR markers in the genome of domestic horse show the result of genetic recombination of different mobile genetic elements (5, 6).

In order to assess the association of ISSR-PCR markers with recombination of retrotransposons, in this work the authors studied genomes of cattle in respect to the fragments homologous to nucleotide sequences previously detected in horse genome.

Technique. The study was performed on genomic DNA of horses the Altai breed and cattle (6 animals of Kazakh Whiteheaded breed and 2 animals of Yakut breed).

Genomic DNA was isolated from the whole blood using DNA-extras-1 kit ("Syntol", Moscow). PCR was performed on a thermocycler Tertsik ("DNA technology", Russia) with RT-PCR kits ("Syntol", Moscow) used according to the manufacturer's recommendations. Amplification of horse DNA was conducted under the regime: primary denaturation (94 °C, 2 min); 30 cycles of denaturation (94 °C, 30 s), primer annealing (62 °C, 30 s), and elongation (72 °C, 2 min); final elongation (72 °C, 10 min). For bovine DNA, this program was modified by reducing the temperature of annealing to 55 °C primer and the number of cycles to 35. The sequences selected as primers were 5'-GAGA-GAGAGAGAGAGAGCCCATGG-3' (leader) and 5'-GAGAGAGAGA-GAGAGCGAAAGAGC-3' (terminator). Electrophoretic analysis of the amplification products was carried out using 1,5% agarose gel in 1½TBE buffer with the addition of ethidium bromide to a final concentration of 0,5 ug/ml and a current of 100 mA and 100 V (time of separation – 1,5 h). The results were visualized with UV-transilluminator UVT-1 ("Biokom", Russia). The size of amplicons was determined using DNA molecular weight marker 100 bp + 1,5 Kb + 3 Kb (12 fragments from 100 to 3000 bp) (M27, "SibEnzyme", Russia).

The mechanisms of formation of DNA regions flanked by inverted sequences were studied in a fragment of 1920 bp from the spectrum obtained in cattle. This fragment was isolated from the agarose gel with DNA Diatom DNA Elution kit ("Isogene", Russia) following the manufacturer's instruction. DNA sequencing was performed in the inter-institutional center of collective use "Genome" of Engelhardt Institute of Molecular Biology, RAS (http://www.genome-centre.ru/, organized with the support of RFBR).

Homologous sequences were found in GenBank by means of BLASTn algorithms (http://blast.ncbi.nlm.nih.gov). Identification of inverted repeats within the sequenced fragment was performed in the program http://mobyle.genouest.org/cgi-bin/Mobyle/, search for homology in the database of repeats - with programs of http://www.repe-atmasker.org/ http://www.girinst.org/censor/.

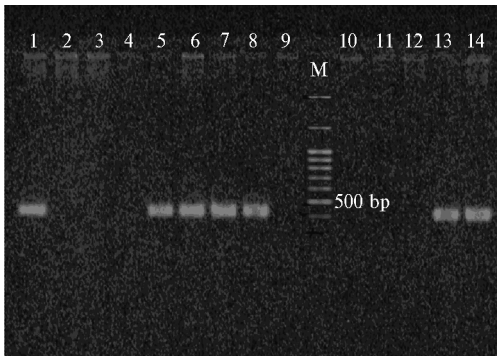

Fig. 1. Amplification products of PCR with horse DNA: M — molecular weight marker DNA 100 bp + 1,5 Kb + 3 Kb (M27, "SibEnzyme", Russia); bands 1, 5, 6, 7, 8, 13, 14 — stallions; bands 2, 3, 4, 9, 10, 11, 12 — mares. Primers: leader — $(AG)_9$C-CCA-TGG, terminator — $(AG)_9$C-GAAAGAGC.

Results. In the earlier research it was found that the horse species-specific amplification product of 416 bp length obtained in the spectrum with the primer $(AG)_9$C may be the result of recombination of evolutionarily old and young mobile genetic elements (5, 6). For this fragment then were developed two primers for PCR: the first one included a part of the initial primer $(AG)_9$C sequence and six nucleotides of the sequenced amplicon of horse (CCATGG), the second primer – a part of the initial primer $(AG)_9$C sequence and eight nucleotides of the sequenced fragment of horse DNA (GAAAGAGC).

PCR with the designed primers resulted in amplification product of the expected length (416 bp) observed in different horse breeds. However, this product wasn't detected in all tested individuals whose DNA was used as template in PCR, but only in stallions (Fig. 1).

These data allowed the assumption that in domestic horse this fragment of DNA is localized in Y-chromosome flanked by the inverted repeat of microsatellite $(AG)_9$ and it was formed through recombination between the ancient DNA transposon (7), an endogenous retrovirus (ERV MLT1G2) (8) first described in fish *Danio rerio* and in human, and retrotransposon of domestic horse ERV1 (9, 10).

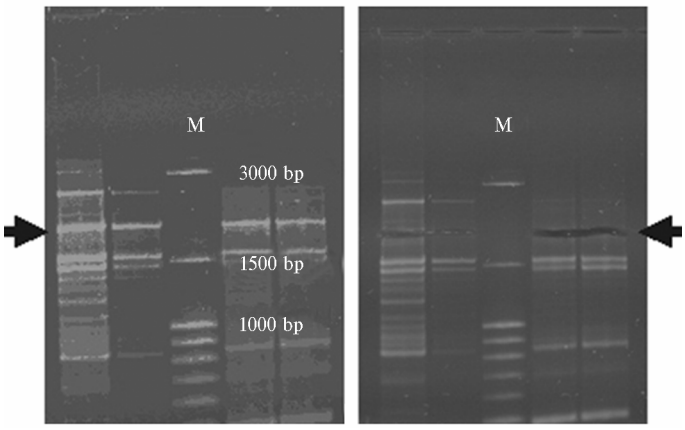

Fig. 2. Amplification products derived from genomic DNA of cattle using the selected pair of primers: M — molecular weight marker DNA 100 bp + 1,5 Kb + 3 Kb (M27, "SibEnzyme", Russia); Arrows point on DNA fragment (left; right – the gel after deletion of the band corresponding to the fragment) further subject to sequencing. Primers: leader— $(AG)_9$C-CCA-TGG, terminator — $(AG)_9$C-GAAAGAGC.

To reveal the presence of this fragment in genomes of other mammals the authors studied samples of cattle DNA derived from animals of Kazakh Whiteheaded and Yakut breeds. In PCR with the same primers as in the case of horse DNA there were obtained complex spectra of amplification products including a number of fragments (Fig. 2). In the cattle DNA, one of these amplicons (arrow, Fig. 2) of 1920 bp length was detected only when using both abovementioned primers in one reaction; it was sequenced and determined the complete sequence with 482 nucleotides on its left flank and 315 nucleotides - on the right flank.

On the left flank of this fragments, by means of programs Repeat-masker and Censor there were identified two endogenous retroviruses of cattle (in positions 6-212 bp – a fragment of ERV1-2-LTR_BT, or LTR/ERV1, the degree of homology more than 94%, 9 transitions, 1 transversion, 4 deletions; in positions 213-416 bp – ERV2-1-I_BT, or LTR / ERVK, more than 70% homology, 28 transitions, 30 transversions, 3 deletions). On its right flank, there was the sequence ERV_classII, or BT-LTR1B, of 305 bp length, 97% homology, 2 transitions, 1 inversion, 7 mononucleotide deletions, and 2 dinucleotide deletions.

Thus, terminal regions of the amplified fragment of cattle genome obtained using the primers based on the flanks of amplified fragment of horse DNA were determined as the result of recombination of endogenous retroviruses Class I and II. It is known that endogenous retroviruses of mammals are assigned to three classes by their origin from exogenous retroviruses, which is estimated from homology of sequences. For example, ERV I Class is assumed to have close relations with retroviruses *Gammaretrovirus* and *Epsilonretrovirus*; for ERV II Class - with *Alpharetrovirus*, *Betaretrovirus*, *Deltaretrovirus* and *Lentivirus*; ERV III Class - with *Spumavirus* (10).

The full-length sequence of 1920 bp cattle amplicon was assessed with BLASTn algorithms to identify homology to both sequenced terminal regions at its left (482 bp) and right (315 bp) flanks. On cattle chromosome 15 there was detected a long sequence of 1921 bp (GenBank: NC_007313.5) whose ends had of 482 bp and 315 bp lengths were 95% homologous to the obtained sequences, so this sequence was assumed as homologous to the abovementioned amplification product (Fig. 2).

Then this full-length sequence was treated with the programs Repeatmasker and Censor to reveal repeats. In position 5 – 938 bp there was found 96% homology to the cattle species-specific endogenous retrovirus BTLTR1B (ERV2) (11), and in position 976 – 1674 bp – more than 70% homology to the sequence of endogenous retrovirus ERV2-1_TSy-I, first described in monkeys *Tarsius tarsier* (12), and in position 1675 – 1921 bp in reverse order the fragment of 256 bp with 92% homology to the sequence of endogenous cattle retrovirus ERV1-2-LTR_BT (ERV1) (13) (Fig. 3).

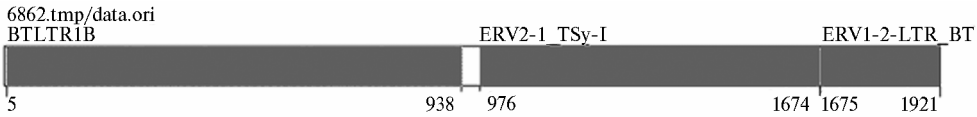

Fig. 3. Distribution of homeologous fragments relative to the fragments of endogenous retroviruses in nucleotide sequence on the fragment of chromosome 15 of cattle (GenBank: NC_007313.5)**.**

These data indicate that the amplification product of cattle DNA 1921 bp length flanked by the primers complementary to the 416 bp fragment of horse genomic DNA is the result of recombination of endogenous retroviruses, as well as the previously shown in horse. In the case of horse, this fragment was formed through recombination between the species-specific endogenous retrovirus ENV1 and more ancient ERV3 first described in humans, while in cattle – the species-specific BTENV1 (*Bos taurus* ENV1) and cattle-specific ENV2 (BTENV2 - *Bos taurus* ENV2) also described in monkeys. So, in horse, in the sequenced fragment a part of the species-specific endogenous retrovirus ENV1 is positioned with the sequence of the endogenous retrovirus first described in primates of different class (in horse - ENV3, in cattle - ENV2).

The link between microsatellites and retrotransposons was traced in many studies. For example, in cattle genome 39% of

microsatellite loci with AGC core are associated with retrotransposon Bov-A2 SINE, evolutionarily young and species-specific for cattle genome (14). Most of retrotransposons were found in introns where predominates the combination of retrotransposon and microsatellite (15).

It is known that a large part of the genetic material, eg. in animals, is presented by retrotransposons: in sequenced genomes of cattle – 46,5% (14), in domestic horse – 36% (16). Polymorphism and species-specificity of retrotransposons, high rate of their diversity during a rapid divergence of living organisms from a common ancestor, were described in laboratory strains of mice (17), which allows a supposition that the main source of genomic variability are mobile genetic elements. The detected matching of flanks in DNA fragments obtained by PCR with primer $(AG)_9C$ in horses and cattle even despite the different lengths of these fragments and participation of the species-specific endogenous retroviruses suggest that ISSR-PCR markers may reflect peculiarities of genomic distribution of microsatellite loci and recombinant products of endogenous retroviruses in genomes of large mammals. Results of these experiments possibly reflect close relations between microsatellites and retrotransposons, as well as the involvement of retrotransposons in complex recombination events in genomes of large mammals.

It should be noted that genomic reproduction of retrotransposons shows undulate dynamics of occurrence, distribution and degradation. For a number of retrotransposons there were described evolutionary cycles that include vertical or horizontal transmission with the outbreak of transpositions and following destruction of most of the initial copies (18). Traces of such cycles are seen as their various multiple residues in chromosomes (5, 19, 20). Mobile genetic elements can move around within the genome with high frequency (as compared to other sources of mutations) at a rate of 10^{-3} to 10^{-5} per element per generation (even to 10^{-2} in certain specific crosses). However, according to the own findings, as well as the available literature data, there persists a certain constancy of close localization and increased frequency of recombination between microsatellite repeats and different endogenous retroviruses.

So, results of studying the amplification products of cattle DNA flanked by inverted repeat of microsatellite with a species-specific motif AG, suggest that in genomes of different animals, particularly in domestic horse and cattle, there are fragments resulting from recombination between sequences of ancient endogenous retroviruses of mammals and evolutionarily younger species-specific retroviruses. Probably, close relations between microsatellites and endogenous retroviruses may underlie high polymorphism in tandem repeats. These data show new opportunities for genome scanning with anchors of microsatellite loci, as well as the fragments of long terminal repeats of endogenous retroviruses.

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