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A swine SINE (PRE-1 sequence) distribution in swine-related animal species and its phylogenetic analysis in swine genome

H Yasue, Y Wada

Summary

The distribution of PRE-1 sequence (a swine SINE) among the animal species related to *Sus scrofa*, i.e. *Phacochoerus aethiopicus* and *Tayassu tajacu*, was examined by dot–blot analysis using PRE-1 sequences as a probe. This revealed that *Phacochoerus aethiopicus* and *Tayassu tajacu* contained PRE-1 sequences, amounts of which in their genomes are almost the same as that in the swine genome, indicating that these species separated after PRE-1 sequences proliferated to diversify in the genome. In order to estimate the time when the PRE-1 started to diversify in the swine genome, PRE-1 sequences were extracted from GenBank DNA database by homology analysis using the PRE-1 consensus sequence as a probe. The 22 PRE-1 sequences obtained were aligned and their phylogenetic relation was calculated by the neighbour-joining method. The result of the calculation combined with the mutation rate of the pseudogenes ($r = 4{\cdot}6 \times 10^{-9}$) indicated that the PRE-1 sequence diversified at least 43·2 million years ago. Taken together, the period of time since the separation of the three species, *Sus scrofa, Phacochoerus aethiopicus* and *Tayassu tajacu*, is currently estimated to be less than 43·2 million years.

Keywords: distribution, PRE-1, swine SINEs

Introduction

Eukaryote genomes contain a large number of repetitive sequences (Britten & Kohne 1968), that consist mainly of tandemly arrayed sequences and interspersed sequences. The interspersed repetitive sequences are classified into two groups: short interspersed repetitive elements (SINEs) and long interspersed repetitive elements (LINEs). The SINEs and LINEs were investigated in various animal species; this revealed that SINEs comprise various kinds of sequences, each of which is conserved only in the animals of related species, whereas LINEs sequences were conserved over a wide range of animal species (reviewed by Weiner *et al.* 1986).

A sequence of SINE in the swine genome was first reported by Singer *et al.* (1987), and named PRE-1. Dot–blot hybridization using the consensus oligonucleotides in the PRE-1 sequences as a probe revealed that the frequency of PRE-1 sequences in the swine genome was 1×10^6 per genome (Takahashi *et al.* 1992). Further analysis on PRE-1 sequences indicated that the progenitor of PRE-1 sequences could be an arginine-tRNA gene, and that the PRE-1 sequences were retroposon. These features are quite similar to those of other SINEs such as human Alu sequences and mouse B2 sequences (Houck *et al.* 1979; Daniels & Deininger 1985; Sakamoto & Okada 1985). In our previous study (Takahashi *et al.* 1992), the PRE-1 sequences were not found in human and bovine genomes, a fact which was also compatible with the general feature of SINEs described above.

In the present study, we examined whether PRE-1 sequences exist in the genomes of animal species related to swine i.e. *Phacochoerus aethiopicus* and *Tayassu tajacu*. To estimate the divergence time of the above animal species, we calculated the phylogenetical time when the PRE-1 sequences diversified in the swine genome based on the PRE-1 sequences registered in the DNA database.

Materials and methods

Preparation of DNA

Genomic DNAs were prepared from spermatozoa or lymphocytes of Large White (*Sus scrofa*), collared peccary (*Tayassu tajacu*), and Holstein–Friesian (*Bos taurus*) as described by Sambrook *et al.* (1989). Blood of the collared peccary was donated by Dr Takaesu (Okinawa Experimental Station of Animal Husbandry, Japan). Genomic DNA of the warthog (*Phacochoerus aethiopicus*) was donated by Dr Imada (National Institute of Animal Health, Japan).

Dot-blot hybridization

Appropriate amounts of swine, warthog, collared peccary, and bovine DNAs were heat-

H Yasue
Y Wada
Animal Genome Research Group, National Institute of Animal Industry, Norin Kenkyu-danchi-Kyoku, Tsukuba, Ibaraki 305, Japan

Correspondence: Hiroshi Yasue.

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denatured and dot-blotted on to a nylon membrane (Boehringer Mannheim, Germany). As a control, *Escherichia coli* DNA was processed exactly as the sample DNAs, and dotblotted. The DNAs on the membrane were hybridized with ^{32}P-labelled probe DNA at 37°C for 40 h in the mixture containing 50% formamide, 2% block solution (Boehringer Mannheim), 5×SSC, 0·1% sodium sarcosineate, 0·04% SDS, 5 mM EDTA, 100 μg/ml denatured herring sperm DNA, and the probe DNA (6×10^5 cpm/ml). For the probe DNA, the Hinc-fragment containing three copies of PRE-1 sequence (Yasue *et al.* 1991) was labelled with ^{32}P using a nick-translation kit (Takara Co., Japan) and denatured. After hybridization, the membrane was washed three times at 25°C with 50% formamide–2×SSC, then twice with 2×SSC. The radioactivity on the resulting membrane was detected with BAS2000 (Fujifilm Co., Japan).

Extraction of PRE-1 sequences from DNA database

Using the PRE-1 consensus sequence reported by Singer *et al.* (1987) as a probe, PRE-1 sequences were extracted from DNA sequences registered in GenBank with the computer software FASTA (Lipman & Pearson 1985).

Construction of the phylogenetic tree of PRE-1 sequences

The PRE-1 sequences were aligned with a computer software CLUSTALW (1·4) (Higgins *et al.* 1992). The phylogenetic distance of the PRE-1 sequences was calculated by the neighbour joining method developed by Saitou & Nei (1987).

Results and discussion

Distribution of PRE-1 sequences among the animal species related to Sus scrofa

In our previous study, PRE-1 sequences were found in the swine genome at a frequency of 1×10^6 per genome and not found in bovine, mouse or human genomes (Takahashi *et al.* 1992). From the viewpoint of animal divergence, the distribution of PRE-1 sequences among the animal species related to *Sus scrofa* is intriguing. Therefore, in the present study, using dot–blot hybridization, we examined whether *Phacochoerus aethiopicus* and *Tayassu tajacu* contain PRE-1 sequences in their genomes. As the results of the dot–blot hybridization shown in Figure 1 demonstrate, the hybridization signals were observed in the DNA samples of Large White, warthog, and collared peccary, and far fewer signals were observed in the bovine or *E. coli* samples. The hybridization results of bovine DNA are consistent with our previous findings (Takahashi *et al.* 1992).

The intensities of hybridization signals were measured and are shown in Table 1. Although the signal intensities in the duplicated samples showed some variation, those of Large White, warthog, and collared peccary were in the same order of magnitude, revealing that warthog and collared peccary contain PRE-1 sequences, the amounts of which in their genomes are similar to that of pigs. These find-

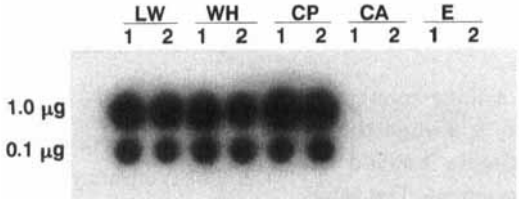

Fig. 1. Dot-blot hybridization using PRE-1 sequences as a probe. One microgram and 0·1 μg DNA of Large White (*Sus scrofa*; LW), warthog (*Phacochoerus aethiopicus;* WH), collared peccary (*Tayassu tajacu;* CP), Holstein–Friesian (*Bos taurus;* CA), and *Escherichia coli* (E) were heat-denatured and dot-blotted on a nylon membrane (Boehringer Mannheim, Mannheim, Germany) in duplicate. The DNAs on the membrane were hybridized with ^{32}P-labelled PRE-1 sequences as described in materials and methods. The hybridized probed DNA was detected with BAS2000 (Fujifilm Co., Japan), and the intensities of the hybridization signals were shown in Table 1.

Table 1. The signal intensity of dot–blot analysis using PRE-1 sequences as a probe

	Intensity of hybridization signals		
	DNA (μg)	Dot 1	Dot 2
Pig	1	$3{\cdot}25\times10^4$	$2{\cdot}58\times10^4$
	0·1	$1{\cdot}05\times10^4$	$7{\cdot}69\times10^3$
Warthog	1	$3{\cdot}67\times10^4$	$1{\cdot}76\times10^4$
	0·1	$1{\cdot}17\times10^4$	$7{\cdot}71\times10^3$
Collared Peccary	1	$3{\cdot}94\times10^4$	$4{\cdot}32\times10^4$
	0·1	$8{\cdot}92\times10^3$	$1{\cdot}23\times10^4$
Cattle	1	430	73
	0·1	nd	nd
Escherichia coli	1	24	nd
	0·1	nd	nd

nd, not detected.

ings further indicate that the evolutionary separation of *Sus scrofa, Phacochoerus aethiopicus,* and *Tayassu tajacu* occurred after the PRE-1 sequences were established as a repetitive sequence in the genome.

Phylogenetical analysis of PRE-1 sequences in the swine genome

In order to estimate the time when the PRE-1 sequences proliferated to diversify in the swine genome, PRE-1 sequences were extracted from the DNA database. The 22 sequences obtained were aligned, and the phylogenetic distances of the sequences were calculated by the neighbour joining method described in Materials and methods (data not shown). Based on this calculation, the phylogenetic tree of the PRE-1 sequences drawn is shown in Figure 2.

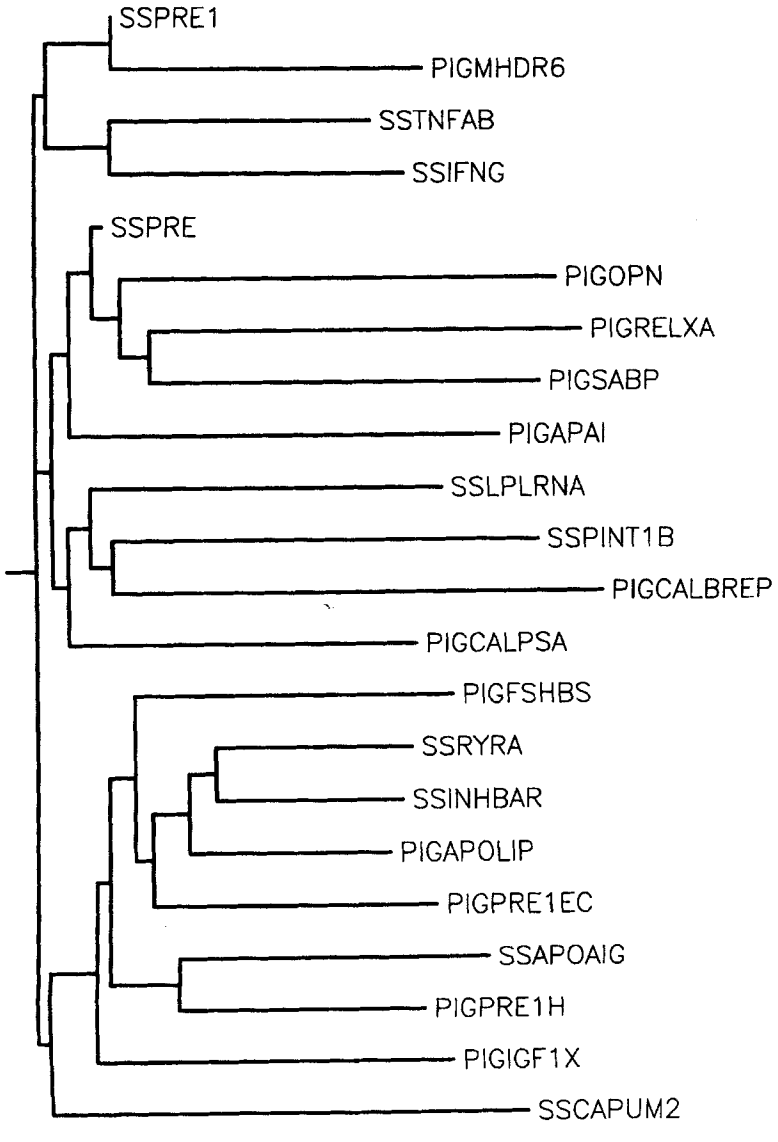

Fig. 2. Phylogenetic distance of 22 PRE-1 sequences. Using the PRE-1 consensus sequence reported by Singer *et al.* (1987) as a probe, PRE-1 sequences were extracted from DNA sequences registered in GenBank with the computer software FASTA (Lipman & Pearson, 1985). The 22 PRE-1 sequences thus obtained were analysed by the neighbor joining method (Saitou & Nei, 1987). The phylogenetic distances of the PRE-1 sequences are shown as a tree structure in this figure.

Of the 22 sequences, the PRE-1 sequence found in the sequence having the locus name SSPRE was indicated to be genetically closest to the PRE-1 ancestry sequence deduced in this calculation. (The PRE-1 sequence was named after the locus in which the sequence was found.) The genetically most-separated sequences were indicated to be PIGOPN and SSAPOAIG. The base change per site between the two sequences was calculated as 0·397. When the base change of PRE-1 sequences is hypothesized to occur at the same frequency as that for pseudogenes ($4{\cdot}6\times10^{-9}$ per year per site) reported by Li *et al.* (1981), it is calculated that the PRE-1 sequences started to diversify at least 43·2 million years ago. Since the number of PRE-1 sequences examined is still small, it seems likely that PRE-1 sequences more heterogeneous than the sequences obtained in the present study could be found in future when many PRE-1 sequences have been registered in the DNA database. This implies that PRE-1 divergence occurred even earlier than our estimation. However, when the findings in the present study are taken together, the period of time since the separation of the three species, *Sus scrofa, Phacochoerus aethiopicus* and *Tayassu tajacu*, is currently estimated to be less than 43·2 million years.

References

Britten R.J. & Kohne D.E. (1986) Repeated sequences in DNA. *Science* **161,** 529–40.

Daniels G.R. & Deininger P.L. (1985) Repeat sequence families derived from mammalian tRNA genes. *Nature* **317,** 819–22.

Higgins D.G., Bleasby A.J. & Fuchs R. (1992) CLUSTAL V: improved software for multiple sequence alignment. *Computer Applications in the Biosciences (CABIOS),* **8,** 189–91.

Houck C.M., Rinehart F.P. & Schmid C.W. (1979) A ubiquitous family of repeated DNA sequences in the human genome. *Journal of Molecular Biology* **132,** 289–306.

Li W.-H., Gojobori T. & Nei M. (1981) Pseudogenes as a paradigm of neutral evolution. *Nature* **292,** 237–9.

Lipman D.J. & Pearson W.R. (1985) Rapid and sensitive protein similarity searches. *Science* **227,** 1435–41.

Saitou N. & Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biological Evolution* **4,** 406–25.

Sakamoto K. & Okada N. (1985) Rodent type 2 Alu family, rat identifier sequence, rabbit C family, and bovine or goat 73-bp repeat may have evoluted from tRNA genes. *Journal of Molecular Evolution* **22,** 134–40.

Sambrook J., Fritsch E.F. & Maniatis T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor, New York.

Singer D.S., Parent L.J. & Ehrlich R. (1987) Identification and DNA sequence of an interspersed repetitive DNA element in the genome of the miniature swine. *Nucleic Acids Research* **15,** 2780.

Takahashi H., Awata T. & Yasue H. (1992) Characterization of swine short interspersed repetitive sequences. *Animal Genetics* **23,** 443–8.

Weiner A.M., Deininger P.L. & Efstratiadis A. (1986) Nonviral retroposons: genes, pseudogenes, and transposable elements generated by the reverse flow of genetic information. *Annual Review of Biochemistry* **55,** 631–61.

Yasue H., Takahashi H., Awata T. & Popescu P.C. (1991) Uneven-distribution of short interspersed repetitive sequence, PRE-1, on swine chromosomes. *Cell Structure and Function* **16,** 475–9.

