Uneven-distribution of short interspersed repetitive sequence, PRE-1, on swine chromosomes.

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ABSTRACT. We investigated the distribution of PRE-1 sequences (a swine major SINE) on the swine chromosomes. The investigation demonstrated that PRE-1 sequences are unevenly distributed along the chromosomes as in the case of the human and mouse SINES. The distribution pattern, however, has no simple correlation with Q-band pattern as that of human and mouse SINES. The prominent difference is as follows; PRE-1 is localized on centromeric regions, but human and mouse SINES are not [KORENBERG and RYKOWSKI (1988). Cell, 53: 391-400; BOYLE, BALLARD, and WARD (1990). Proc. Natl. Acad. Sci. U.S.A. 87: 7751-7761].

Repetitive sequences in mammalian genomes are classified generally into two groups: one is satellite sequences, and the other, interspersed repetitive sequences (15, 16). The latter was further divided to subgroups primarily by their size; those are short interspersed sequences (SINES) and long interspersed sequences (LINES). These repetitive sequences have been utilized for characterization of chromosome (1, 8, 17, 20), analysis of chromosome organization (1, 6, 7, 10), and gene mapping (5, 13). The analyses for the human major SINES (Alu) and LINES (L1) have shown that Alu and L1 were preferentially localized in the R-positive band regions (generally corresponding to Q-negative band regions) and in Q-positive band regions, respectively. From taking into account the findings that the R-positive regions replicate early in the DNA synthetic period and the G/Q-positive regions replicate late, it is surmised that the SINES and LINES may play roles in chromosome organization including replication and condensation (10). This hypothesis is supported by a similar observation for the mouse SINES (B1 and B2) and LINES (L1) (1).

In our investigation on swine repetitive sequences, we have molecularly-cloned the swine DNA segment (named Hinc-fragment) which contains three PRE-1 sequences, the major SINE of the swine genome (19). When this fragment was used as a probe for hybridization of swine genomic DNA, almost all the hybridization signals were found to be attributed to the three PRE-1 sequences. Therefore, we attempted to investigate the distribution of PRE-1 sequences along the chromosomes using Hinc-fragment as a probe, not only for the characterization of swine chromosomes but also for the examination of whether the distribution pattern of PRE-1 sequences resembled to those of the human and mouse SINES.

MATERIALS AND METHODS

Preparation of chromosome-spreads on glass-slides. Peripheral blood cells from pigs (Large White) were cultured in RPMI1640 containing 10% fetal calf serum and 10 μl/ml phytohemagglutinin (HA-15; Wellcome Diagnostics Co. UK) for 43 hr. Colcemid was added to a final concentration of 0.04 μg/ml 30 min before ending the culture. The cultured cells were treated with hypotonic solution and fixative, then spread on glass-slides (21).

Q-banding of chromosomes. The chromosome-spreads on glass-slides were stained with quinacrine mustard according to the methods as described by Caspersson et al. (2), and photographed.

Probe DNA for in situ hybridization. In our investigation on swine repetitive sequences, the swine genomic DNA segment (Hinc-fragment) which was supposed to contain repetitive sequences was molecularly-cloned, then sequenced according to the dideoxy chain termination method (18) (Fig. 1). When compared with the swine sequence stored in GenBank, Hinc-fragment was found to contain three PRE-1 sequences (shown with box in Fig. 1) which had been reported as swine SINES by Singer et al. (19). Most parts of the sequence (shown with solid underline in Fig. 1) in Hinc-fragment except the PRE-1 sequences were found to have low repetitive frequencies in the swine genome (~10 copies/haploid genome). The remaining sequences [-26 base pairs (bp) long] (shown...
Fig. 1. Nucleotide-sequence of Hinc-fragment used as a probe DNA for in situ hybridization. Sequences in the box are PRE-1 sequences (19); sequences with solid underline were examined for their repetitive frequencies in the swine genome (see text); and sequences with broken line were not examined for their repetitive frequencies in the genome.
with broken underline in Fig. 1), the repetitive frequencies of which have not determined, are not able to hybridize even with the homologous sequences under the hybridization conditions described below. Thus, we decided to use Hinc-fragment as a probe DNA for the investigation on the distribution of PRE-1 sequences along the chromosomes.

For probe DNA of in situ hybridization, pBluescript (pBS; Stratagene Co. USA) containing swine Hinc-fragment (pBS-Hinc) and, as a control, pBS containing no insert were prepared in the form of single strand DNA, and labeled with biotin according to the method of Forster et al. (4). The specific activity of the probe DNAs was confirmed to be similar, prior to their use for hybridization.

In situ hybridization. The chromosome-spreads on glass-slides were treated with RNase and alkali for in situ hybridization as described previously (21). The resulting spreads were subjected to the in situ hybridization using following conditions: The hybridization mixture contained 2 μg/ml probe DNA, 250 μg/ml M. lysodeikticus DNA as a carrier, 6× SSC, 10 mM EDTA, 5× Denhardt, 45% formamide. One hundred microliters of the mixture was put on the one glass-slide and covered with siliconized coverslip, followed by incubation at 39°C for 15 hr. After hybridization, the glass-slides were washed with 2× SSC containing 50% formamide at 30°C and with 2× SSC, followed by dehydration in ethanol. The biotin on the resulting glass-slides was detected using alkaline phosphatase-avidin/BCIP/NBT system and photographed with a 595 nm band-pass filter (22).

**RESULTS AND DISCUSSION**

In situ hybridization. Swine chromosome-spreads on glass-slides were subjected to in situ hybridization using pBS-Hinc as a probe. More than 250 cells in metaphase were inspected with microscope and the cells displaying well-separated chromosomes were photographed. A representative chromosome-spread is shown in Fig. 2A, in which hybridization signals are unevenly distributed along the chromosomes. When the chromosomes were probed with PBS as a control experiment, no hybridization signals resulted on the chromosomes (Fig. 2B). These results together with the fact that almost the same signal patterns were observed on each of the sister chromatids strongly indicate that the signals are the result of the hybridization of the PRE-1 sequences of Hinc-fragment to chromosomal DNA. [The PRE-1 sequences of Hinc-fragment would hybridize with a portion of PRE-1 family in the genome because of their sequence deviation up to 30% from the consensus sequence (unpublished data), so we describe the PRE-1 sequences detected with Hinc-fragment as "PRE-1"]. Compared with the distribution pattern of human and mouse SINES on chromosomes reported (1, 10), the "PRE-1" distribution pattern seems to be differ-
ent from especially in that "PRE-1" are preferentially localized on the centromeric region of chromosomes.

Relationship between "PRE-1" distribution pattern and Q-band pattern. Since Alu, B1, and B2 distribution patterns have been described based on the R-band pattern, the precise comparison of "PRE-1" distribution with Alu, B1, and B2 necessitates a clarification of the relationship between "PRE-1" distribution pattern and G/Q or R-band pattern (1, 10). To clarify the relationship, the cells in metaphase were first Q-banded, then subjected to the in situ hybridization, followed by comparison of Q-band pattern with the hybridization signal pattern. For the comparison, the brightness of the Q-band and the strength of the signal were expressed with the respective brightness of the colors red and green (Fig. 3A and 3B); and both images were superimposed using the image analyzer (Fig. 3C). The relationship was judged from the tint of red and green colors on the superimposed image. Y-chromosome shown in the inset of Fig. 3C displayed a distinct and simple segregation of red and green colors, indicating that "PRE-1" sequences were preferentially localized on the Q-negative region, preferentially on the long arm of the chromosome. Characterizing the remaining chromosomes was, however, not as simple for Y-chromosome; they displayed no simple segregation of both colors, and most of them contain red, green, yellow, and dark colors. This indicated that these chromosomes comprise "PRE-1"-dense/Q-positive, "PRE-1"-dense/Q-negative, "PRE-1"-sparse/Q-positive, and "PRE-1"-sparse/Q-negative regions. Consequently, the "PRE-1" distribution pattern appears to have no simple correlation with Q-band pattern in any but Y-chromosome.

As mentioned above, Alu, B1, and B2 are localized preferentially on the regions of R-positive band. On the other hand, the Q-negative regions in swine chromosomes except Y correspond to R-positive bands (3), and "PRE-1" is localized preferentially on the long arm of Y-chromosome where no R-positive band exists, so that, "PRE-1" distribution pattern on the chromosomes appears to have no specific nor simple correlation with R-positive band. Therefore, in terms of this matter, "PRE-1" is localized on the chromosomes in a different fashion from Alu, B1, and B2.

The distribution pattern of Alu was first revealed...
using a single Alu sequence (BluR8) as a probe (10), then confirmed by the Alu consensus sequence (14). These facts indicate that the distribution pattern reported would reflect that based on whole members of Alu family, though Alu family shows up to 28% deviation from consensus sequence (9). B1 and B2 sequences are shown to display a deviation of no more than 5% (11, 12). Hence, the B1 and B2 distribution patterns reported are likely to reflect the patterns based on the whole members of the B1 and B2 families, respectively. However, the “PRE-1” distribution pattern, as mentioned above, is likely to represent that of PRE-1 sub-family, though the present study used three PRE-1 sequences as a probe. Nevertheless, when one takes into account the studies on Alu distribution, it is possible that the “PRE-1” distribution pattern coincides with the pattern based on the whole members of PRE-1 family.

If we assume that the “PRE-1” distribution pattern coincides with the distribution pattern based on the whole members of PRE-1 family, the distribution pattern of PRE-1 family on the chromosomes is quite different from those of Alu, B1, and B2 families. In addition, this may cast a question to the hypotheses about the roles of SINES proposed by Korenberg and Rykowski (10).

REFERENCES


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