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Analyses of prevalence and polymorphisms of six replication-competent and chromosomally assigned porcine endogenous retroviruses in individual pigs and pig subspecies

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Abstract

As porcine endogenous retroviruses (PERV) productively infect human cells in vitro, they pose a serious risk in xenotransplantation and xenogeneic cell therapies. We have analyzed the prevalence of six well-characterized full-length PERV, five of them being replication-competent and four of them being chromosomally assigned (J. Virol. 75 (2001) 5465; J. Virol. 76 (2002) 2714). These analyses revealed a heterogeneous distribution of PERV among individuals and, as no PERV is present in every pig, it seems feasible to generate pigs free of functional PERV by conventional breeding. Conversely, as PERV are polymorphic, single proviruses may have escaped detection and this kind of assay must be performed for every herd used in xenotransplantation or xenogeneic cell therapies. In addition, specific proviruses show internal point mutations which significantly affect their replicational capacities. As there are two different types of PERV LTR structures showing varying levels of transcriptional capacity (J. Virol. 75 (2001) 6933), an analysis of 21 distinct chromosomal locations revealed that PERV which harbor highly active LTRs with repeat elements in U3 are dominant.

Keywords: PERV; Replication-competence; Chromosomal allocation; Polymorphisms

Introduction

The therapeutic use of animal cells, tissues, and organs derived from pigs as donors in the course of xenotransplantations (XTx) and xenogeneic cell therapies might help to overcome the growing shortage of allotransplants from humans.

Major concerns have been raised regarding infectious risks posed by the possibility of introducing new agents from the animal into the recipient, leading to xenozoonosis (Fishman, 1994, 1997; Hunkeler et al., 1999; Michaels and Simmons, 1994; Stoye and Coffin, 1995). Many methods used to remove infectious agents (e.g., vaccination) are not appropriate to avoid the presence of endogenous retroviruses (ERV) which are transmitted vertically in the germ line.

Porcine endogenous retrovirus (PERV) released from porcine cells infect human cells in vitro (Martin et al., 1998; Patience et al., 1997; Wilson et al., 1998, 2000). The risk of xenozoonosis is even enhanced if genetically engineered pigs which are produced to reduce the host-versus-graft reaction are used (Bach et al., 1995; Sandrini et al., 1995; Weiss, 1998).

A retrospective study revealed no transmission of PERV to patients treated with pig tissue (Paradis et al., 1999). However, in an NOD/SCID mouse model, the diabetic and immunodeficient animals showed infection with and expression of PERV in different tissues after xenotransplantation of porcine islet cells, suggesting that PERV are xenozoonotic in vivo (Van der Laan et al., 2000).

Approximately 30–50 integration sites of PERV exist in the genome of different pig breeds (Akiyoshi et al., 1998; LeTissier et al., 1997) and three classes of infectious endogenous gammaretroviruses (PERV-A, PERV-B, and PERV-C) are known (Takeuchi et al., 1998; Patience et al.,

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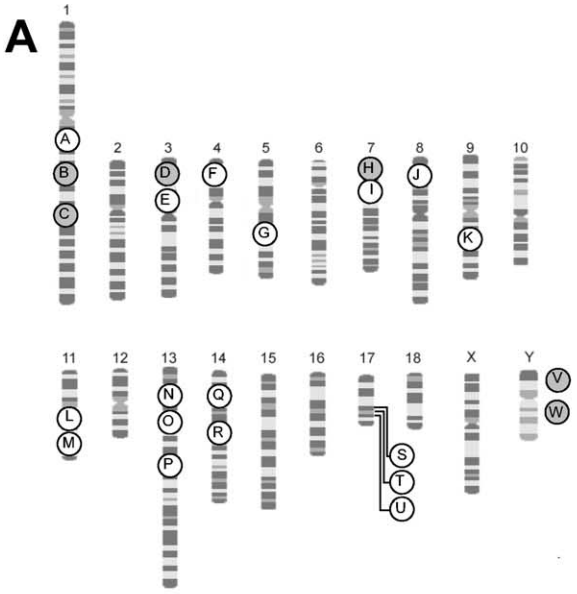

B

	Clone	Position	Class	LTR structure
A	141G12	1q2.1	A	w/o repeat
B	130A12	1q2.4	A	w/o repeat
C	151B10	1q2.3	A	w/o repeat
D	463H12	3p1.5	A	2 ½
E	258A11	3p1.5	A	1 ½
F	161B7	4p1.1	B	2 ½
G	305F5	5q2.3	A	w/o repeat
H	192B9	7p1.1	B	3 ½
I	484G4	7p1.2	B	1 ½
J	135E5	8p1.2	A	2 ½
K	783D7	9q2.6	B	1 ½
L	1058D6	11q1.2	B	2 ½
M	647G4	11q1.4	B	1 ½
N	253B6	13q4.3	A	w/o repeat
O	383E10	13q4.3	A	2 ½
P	242D4	13q4.9	A	w/o repeat
Q	498D8	14q1.1	B	2 ½
R	80H6	14q1.4	B	1 ½
S	1079D9	17q1.2	A	2 ½
T	667G4	17q2.1	B	1 ½
U	534G4	17q2.1	B	3 ½
V	PK15-(58)	???	A	w/o repeat
W	PK15-(213)	???	B	2 ½

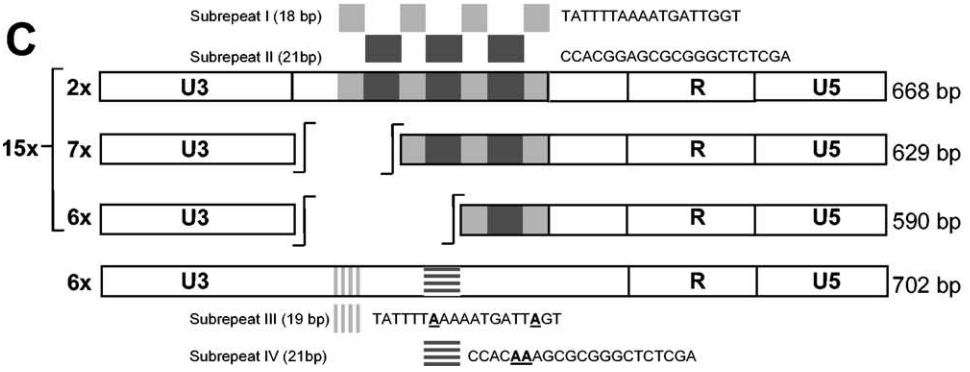

Fig. 1. (A) Porcine karyogram of chromosomally assigned intact or mostly intact porcine endogenous retroviruses. Replication-competent proviruses are indicated by gray circles. (B) Summary of chromosomal positions in a "*large white*" pig genomic library represented by BAC clones and analyzed for proviral PERV. Localization of molecularly characterized PERV proviruses is indicated by gray dots and boldface letters. B, 151B10 @ 1q2.3; C, 130A12 @ 1q2.4; D, 463H12 @ 3p1.5; H, 192B9 @ 7p1.1; V and W, PK15-PERV-A(58) and PK15-PERV-B(213), respectively; both clones are not chromosomally assigned. Other proviral positions shown represent mostly intact but replication-deficient clones (Rogel-Gaillard et al., 1999; Niebert et al., 2002) whose LTRs were used for comparison. The flanking sequence of clone 192B9 maps to BAT1 located within the porcine SLA complex on chromosome 7. (C) Structure of LTR of proviral PERV identified in 21 BAC clones of a *large white* pig genomic library. Fifteen LTRs with a distinct repeat pattern in U3 display elevated levels of promoter activity in luciferase reporter assays, whereby transcriptional strength increases with the number of subrepeats I and II (Scheef et al., 2001). The repeatless LTRs found in 6 proviruses of PERV display a significantly lower transcriptional activity in luciferase reporter assays (Scheef et al., 2001). Although sequences homologous to subrepeat I and II are present, these sequences denoted as subrepeat III and IV are not organized in a continuous pattern and display slight sequence variations, indicated by bold face letters in the corresponding sequences.

2001). These classes display high sequence homology in the genes coding for the group-specific antigens (Gag) and the polymerase (Pol) but differ in the genes encoding the envelope proteins (Env) which determine the different host ranges of the classes (LeTissier et al., 1997; Takeuchi et al., 1998; Patience et al., 2001). We have previously reported the isolation and characterization of replication-competent molecular clones of PERV derived from infected human cells (Czauderna et al., 2000) and from two porcine sources (Krach et al., 2001; Niebert et al., 2002). The findings allow the comparison of functional PERV from different origins directly on the molecular and cellular level and mapping of these proviral sequences to chromosomal locations of one specific pig breed (Fig. 1A and B). Based on the knowledge of the number and location of functional PERV we have screened several individuals of different pig breeds to determine the prevalence and variability of PERV. In addition, the analysis demonstrates the possibility of generating pigs free of functional PERV by conventional breeding.

Results and discussion

Prevalence of PERV

As the proviruses described previously are derived from the genome of *large white* pigs (Krach et al., 2001; Niebert et al., 2002), we initially analyzed a number of these ani-

mals for the presence or absence of specific PERV using primers derived from chromosomally flanking sequences unique for each single provirus. Different individual *large white* pigs revealed a heterogenous distribution of specific PERV as summarized in Table 1a and b. There is no particular PERV present in every pig; however, some proviruses are observed more often than others. Bac-PERV-A(130A12) is observed in 14 of 37 individuals while the other figures are 14 of 37 (Bac-PERV-A(151B10)), 8 of 37 (Bac-PERV-A(463H12)), 31 of 37 (Bac-PERV-B(192B9)), 26 of 37 (PK15-PERV-A(58)), and 20 of 37 (PK15-Perv-B(213)), respectively. All but one provirus (Bac-PERV-A(463H12)) are present in the porcine cell line PK15 (Table 1a, sample 77). This result suggests that careful selection of founder animals allows for elimination of replication-competent PERV from a herd designated for XTx by conventional breeding. While functional PERV could be eliminated that way, numerous remaining open reading frames of defect PERV (Fig. 1A and B) are cumbersome to be removed and may be able to recombine with other infectious agents in a XTx scenario. Conversely, the heterogeneous distribution of PERV could bias the identification of replication-competent PERV by the initial choice of genetic material (Krach et al., 2001; Niebert et al., 2002) and could have missed single PERV not present in that material. Therefore, new analyses should be performed on every pig herd designated for XTx. In any case, as further analyses reveal (see below), the proviral integration sites seem to be conserved at large in different pig breeds.

As the two proviral PERV identified by Krach et al. (2001) could not be chromosomally assigned (see below), the correlation with PERV identified in the BAC library of a *large white* pig (Niebert et al., 2002) was analyzed. Different patterns of distribution as well as different chromosomal flanking sequences show that the proviruses are distinct from each other (Table 1a).

For clone PK15-PERV-B(213), two single LTRs (Table 1a, samples 3 and 5) were identified. Sequencing and BLAST search confirmed that both LTRs belong to this provirus. This result indicates that PERV are genetically active and that transposition events are likely to happen. Furthermore, differences present in pig subspecies were analyzed (Table 1a and b, Fig. 2; see below).

Localisation of proviruses

Chromosomal mapping of the two clones isolated from porcine cell line PK15 was attempted using a *large white* pig genomic library (Rogel-Gaillard et al., 1999) and a somatic cell hybrid panel (Chevalet et al., 1997). However, no clear results were revealed, which might be due to excessive rearrangements in the chromosomal structure as shown for other cell lines (Harris et al., 2003; Hwang et al., 2003), whereby cell line PK15 was established in the laboratory a long time ago (Todaro et al., 1974). In addition, PERV could induce chromosomal changes on its own by reinfection and insertional mutagenesis as demonstrated for other viruses (Hughes and Coffin, 2001; Livezey et al., 2002; Miller et al., 2002) or the proviruses simply were not present in the porcine material used to generate the *large white* library or cell hybrid panel, respectively. As the chromosomal position of the other four proviruses is known (Rogel-Gaillard et al., 1999; Fig. 1A and B), we tried to assess the insertional positions on a gene-specific basis, but only succeeded for clone PERV-B(Bac-192B9). A BLAST analysis with the chromosomal flanking sequence revealed an integration into the BAT1 gene (Peelman et al., 1995) with 100% confidence. BAT1 is located within the porcine SLA complex on chromosome 7 and codes for a putative conserved RNA helicase of the DEAD family. The location of BAT1 on chromosome 7 is consistent with the earlier mapping of PERV-B(Bac-192B9) on the same chromosome (Rogel-Gaillard et al., 1999). All other proviruses could not be assigned closer to known gene positions as available information on the porcine genome is not comprehensive.

Intraviral polymorphisms

Clone Bac-PERV-B(192B9) which displayed two in-frame stop mutations within the *pol* gene (Niebert et al., 2002) was investigated for intraviral polymorphisms in individual pigs. Amplification of the *pol* gene of Bac-PERV-B(192B9) using 13 different samples from *large white* pigs and miniature pigs (Table 1a, samples 25–37) revealed that these mutations are reverted in 8 of 13 animals (Fig. 3), one of the two point mutations is reverted in 4 of 13 samples, and 1 sample displayed both mutations (data not shown). We assume that proviruses derived from one of the eight animals displaying an intact *pol* open reading frame are capable of productive infection, as the sequence homology is $>$99% to replication-competent clone 293-PERV-B(43) (Czauderna et al., 2000). However, no experimental evidence is provided yet. This example shows, as proviruses tested to be defective in one assay (Fig. 1A and B; Rogel-Gaillard et al., 1999; Niebert et al., 2002) based on limited genomic material from one individual pig, other PERV may show replicative function if enough or different samples are investigated.

Differences in subspecies

Different pig subspecies could serve for XTx applications because of varying needs in size and physiological compatibility or due to genetic engineering. Therefore, we wanted to know whether sequences derived from *large white* pigs are useful for screening other breeds and whether PERV are distributed equally in every subspecies or whether there are differences and, consequently, a specific breed should be favored because of fewer PERV integration loci. For a number of reasons, there is a focus on minipigs in XTx applications. We have studied DNA from these animals in addition to *large white* pigs. While two miniature

Table 1a
Presence of six PERV in individuals of different pig subspecies

	PERV-A(Bac-130A12)	PERV-A(Bac-151B10)	PERV-A(Bac-463H12)	PERV-B(Bac-192B9)	PK15-PERV-A(58)	PK15-PERV-B(213)
1 Large white RKI-U247	+	−	−	+	−	−
2 Large white RKI-U245	+	−	−	+	+	+
3 Large white RKI-U235	+	+	−	+	+	Single LTR
4 Large white RKI-U232	+	+	−	−	+	−
5 Large white RKI-U229	−	+	−	+	+	Single LTR
6 Large white RKI-U222	−	+	−	+	+	+
7 Large white RKI-U093	+	−	−	−	+	+
8 Large white RKI-U087	+	+	−	+	+	+
9 Large white RKI-U292	−	+	−	−	+	+
10 Large white RKI-U291	−	+	−	+	+	+
11 Large white RKI-U280	−	+	−	−	+	+
12 Large white RKI-U272	−	−	+	+	+	−
13 Large white RKI-U267	−	+	−	+	+	+
14 Large white RKI-U265	+	+	−	+	+	+
15 Large white RKI-U257	−	+	−	+	+	+
16 Large white RKI-U256	+	−	−	+	+	+
17 Large white RKI-U324	−	−	+	+	+	−
18 Large white RKI-U323	−	−	+	+	+	+
19 Large white RKI-U314	−	−	−	+	+	−
20 Large white RKI-U312	−	+	−	+	+	+
21 Large white RKI-U303	−	−	−	+	+	+
22 Large white RKI-U300	−	−	−	+	+	+
23 Large white PEI 1	−	−	−	−	+	+
24 Large white PEI 2	−	+	−	−	−	−
25 Large white PEI 3	+	−	−	+	−	−
26 Large white PEI 4/5	+	−	−	+	−	−
27 Large white PEI 94	−	−	+	+	+	+
28 Large white PEI 95	+	+	−	+	+	+
29 Large white PEI 97	−	−	+	+	−	−
30 Large white PEI 100	−	−	+	+	−	−
31 Large white PEI 101	+	−	−	+	−	−
32 Large white PEI 105	−	−	−	+	−	−
33 Large white PEI 106	+	−	−	+	−	−
34 Large white PEI 013	−	−	+	+	−	−
35 Large white PEI 023	−	−	−	+	−	−
36 Miniature pig 1	+	−	−	+	+	+
37 Miniature pig 2	−	−	+	+	+	+
38 d/d Minipig 369	−	−	−	−	−	−
39 d/d Minipig 370	−	−	−	−	−	−
40 d/d Minipig 371	−	−	−	−	−	−
41 d/d Minipig 379	−	−	−	−	−	−
42 d/d Minipig 380	−	−	−	−	−	−
43 d/d Minipig 385	−	−	−	−	−	−
44 d/d Minipig 392	−	−	−	−	−	−
45 d/d Minipig 393	−	−	−	−	−	−
46 d/d Minipig 397	−	−	−	−	−	−
47 d/d Minipig 398	−	−	−	−	−	−
48 d/d Minipig 399	−	−	−	−	−	−
49 d/d Minipig 403	−	−	−	−	−	−
50 d/d Minipig 404	−	−	−	−	−	−
51 d/d Minipig 405	−	−	−	−	−	−
52 d/d Minipig 406	−	−	−	−	−	−
53 Wild boar 553	+	−	−	−	−	−
54 Wild boar 558	+	−	+	−	−	+
55 Wild boar 561	−	+	−	−	−	−
56 Wild boar 564	−	−	−	−	−	+
57 Wild boar 569	−	−	−	−	−	+
58 Wild boar 579	−	−	−	+	−	−
59 Wild boar 533	−	−	−	−	−	−
60 Wild boar 536	−	−	−	−	−	−
61 Wild boar 550	+	−	+	−	−	−
62 Wild boar 584	−	−	−	+	+	−
63 Wild boar 538	−	+	−	−	−	−
64 Wild boar 547	+	−	+	−	+	−
65 Wild boar 544	−	+	−	−	−	+
66 Wild boar 541	−	−	−	−	+	+
67 Westran pig 1	−	−	−	−	−	+
68 Westran pig 2	−	+	−	−	−	+
69 Westran pig 3	−	−	−	−	−	−
70 Westran pig 4	−	−	−	−	+	−
71 Westran pig 5	+	−	−	−	+	+
72 Westran pig 6	−	−	−	−	−	−
73 Westran pig 7	+	−	−	−	+	−
74 Westran pig 8	−	−	−	−	−	−
75 Westran pig 9	−	−	−	+	−	−
76 Westran pig 10	−	−	−	+	−	−
77 PK15 cell line	+	+	−	+	+	+

Note. Results of PCR analyses of specific proviruses using genomic DNA of different individuals of five pig subspecies. + Presence of provirus; −, absence of provirus; single LTR indicates the absence of the provirus; instead a PERV LTR indicating an excised provirus was identified by sequencing. Numbers given as a suffix to each sample have been denoted according to the supplier's nomenclature. Note that 15 minipigs (samples 38 to 52) marked by the prefix d/d are descendants of miniature pigs, but were inbred for an extended period of time to generate a defined SLA haplotype (Kaeffer et al., 1990, 1991). Most individuals of this breed are described as nontransmitters of PERV (Oldmixon et al., 2002).

Table 1b
Prevalence of six PERV on a per-species basis

	PERV-A(Bac-130A12)	PERV-A(Bac-151B10)	PERV-A(Bac-463H12)	PERV-B(Bac-192B9)	PK15-PERV-A(58)	PK15-PERV-B(213)
Large white	13/35	14/35	7/35	29/35	24/35	18/35
Miniature pig	1/2	0/2	1/2	2/2	2/2	2/2
Westran pigs	2/10	1/10	0/10	2/10	3/10	3/10
Wild boar	4/14	3/14	3/14	2/14	3/14	5/14
d/d Minipig (France)	0/15	0/15	0/15	0/15	0/15	0/15

Note. Summary of the results given in Table 1a. Samples tested positive for the respective provirus are given as a fraction of the overall number of tested samples.

pig samples from our in-house breed indicated that *large white* derived sequences can be used to screen for PERV in different breeds (Table 1a and b, samples 36 and 37), none of the six PERV can be identified in any sample from Yucatan minipigs bearing the d/d haplotype, i.e., homozygous for the SLA class II antigens (Kaeffer et al., 1990, 1991; Oldmixon et al., 2002). Individuals of this highly inbred subspecies have been described as nontransmitters of PERV (Oldmixon et al., 2002). Thus, the lack of production of infectious virions could obviously be attributed to an absence of replication-competent PERV (Table 1a and b, samples 38–52).

In addition to miniature pigs and inbred d/d minipigs, we have investigated German wild boars and Australian *Westran* pigs. Both German wild boars and *Westran* pigs are free-roaming animals in their natural habitats with little or no level of inbreeding. The *Westran* pigs in particular are endemic in a small part of Australia, released by farmers approx. 200 years ago. Surprisingly, the prevalence of specific PERV in wild boars as well as *Westran* pigs is much lower than in industrially bred *large white* pigs (Table 1a and b, samples 1–35, 53–66, 67–76). This observation is consistent with a recent publication describing that the number of proviral PERV increases in closed herds (Mang et al., 2001). The *Westran* pigs illustrate the influence of founder effects in the initial choice of the herd, as no PERV-A(Bac-463H12) could be detected in these animals. The differences in subspecies given in Table 1a for individuals are summarized in Table 1b on a per-species basis for better comparison.

Frequency of different LTR structures

Our recent investigations revealed the presence of two different LTR structures (Fig. 1C) in PERV giving rise to largely different replicative activities (Scheef et al., 2001, 2002). In addition, viruses bearing a structured LTR with distinct 39-bp repeats in U3 can adapt their transcriptional properties in response to the host cell type by increasing or decreasing the number of repeats. In cell culture this gives rise to LTRs with different numbers of repeat elements, even some with excessive high repeat counts (>5) displaying very high replicative performance and cell toxicity (Scheef et al., 2001). Depending on the respective host cell line a preferred sequence with an intermediate repeat number is selected (Scheef et al., 2001). To investigate whether these LTRs also occur in vivo we have analyzed the LTRs in 21 distinct BAC clones to avoid duplication of LTRs when performing this analysis on genomic DNA.

The frequency of LTR structures found within the library of a *large white* pig clearly shows a dominance of LTRs harboring repeat elements in U3 (15 to 6), summarized in Fig. 1C. Therefore, we assume that LTRs bearing repeats had a selective advantage as they are present in the majority

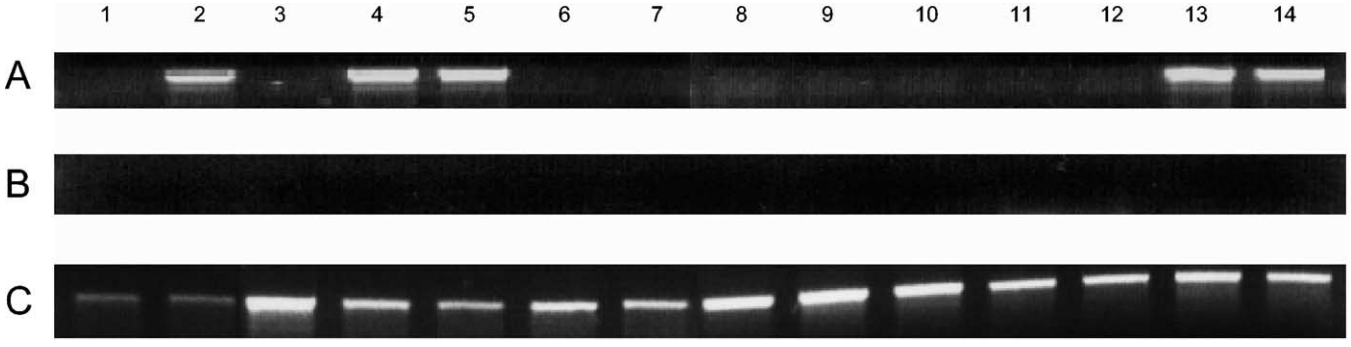

Fig. 2. Sample analyses of detection of specific PERV proviruses in genomic DNA of different pig subspecies by PCR using primers derived from flanking chromosomal sequences. (A) Detection of provirus PK15-PERV-B in genomic DNA of wild boars (samples 53–66). (B) Failed detection of provirus PK15-PERV-B in genomic DNA of d/d minipigs (samples 38–51 are shown). (C) Amplification of ubiquitous ADP ribosylation factor 3 (ARF-3) gene in genomic DNA of d/d minipigs (samples 38–51 are shown).

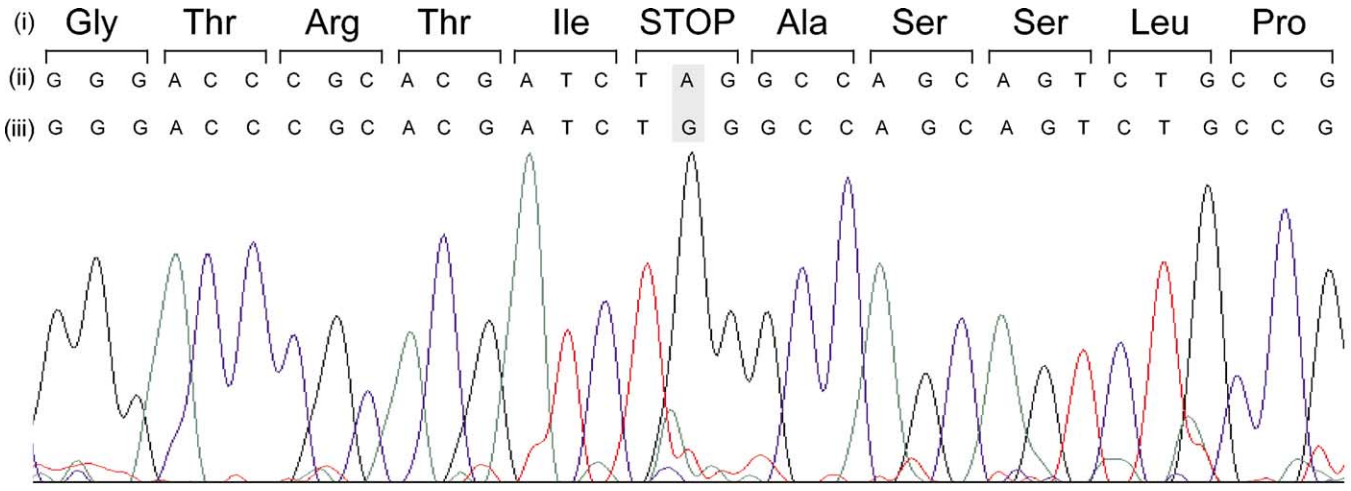

Fig. 3. Display of exemplary chromatogram of reverted point mutation in the *pol* open reading frame of clone Bac-PERV-B(192B9) at position 4687 (gray box). Line (i) indicates the amino acid sequence of PERV-B(Bac-192B9) Pol as it was found in the BAC library and line (ii) gives the corresponding nucleotide sequence; line (iii) shows the nucleotide sequence of sample 28, where the stop codon is reverted to tryptophan (Trp).

of proviruses. The distribution of repeat motifs is similar to the data revealed by previous cell culture experiments (Scheef et al., 2001). While LTRs with many repeats are generated rapidly in cell culture, no LTR with extensive repeat numbers is present in vivo and only two LTRs with a maximum of 3.5 repeats were identified. The majority of LTRs (13 of 21) displays 1.5 or 2.5 repeats which show intermediate promoter activities in reporter gene assays mediated by transcription factor NF-Y (Scheef et al., 2001, 2002). This intermediate activity might be the best compromise between virus replication and cell survival (Bonhoefer and Sniegowski, 2002). An extended analysis to determine the age of PERV employing a molecular-clock approach is pending (manuscript in preparation).

The human cell line 293 was infected with PERV by cocultivation with PK15 cells (Patience et al., 1997) yielding cell line 293-PERV-PK which was subsequently used to isolate PERV proviruses for the first time (Czauderna et al., 2000). Three proviruses were isolated with all of them harboring structured LTRs. In addition, the amplification and subsequent sequencing of PERV LTR fragments from genomic DNA of PERV-infected human cell line 293-PERV-PK only revealed LTRs harboring the distinct repeat structure in varying configurations, but no repeatless LTRs (data not shown). Thus, it might be possible that proviruses harboring repeatless LTRs, although capable of minor replication when artificially introduced into cell culture, might not be able to cross the species barrier according to native conditions.

Materials and methods

Construction of porcine genomic libraries

The procedures used to generate and screen the porcine genomic BAC library were described previously (Rogel-Gaillard et al., 1999; Niebert et al., 2002). The isolated clones are designated Bac-PERV-A(130A12), Bac-PERV-B(192B9), Bac-PERV-A(151B10), and Bac-PERV-A(463H12).

Generation and screening of a bacteriophage λ library of porcine cell line PK15 have been described recently (Krach et al., 2001). The clones derived thereof are designated PK15-PERV-A(58) and PK15-PERV-B(213).

Inverse PCR

Inverse PCR experiments were performed as described previously (Czauderna et al., 2000) to reveal provirus-specific chromosomal sequences. These flanking sequences of PERV proviruses needed to perform appropriate assays have been summarized elsewhere (Patent No. 101114338, German patent office).

Detection of proviral sequences in genomic DNA samples

To detect specific chromosomally assigned proviral PERV, PCR was performed on porcine genomic DNA using a PCR program as follows, 94°C for 3 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and elongation at 72°C for 1.5 min, followed by a final elongation step at 72°C for 20 min.

Analysis of specific proviruses was enabled by use of PCR primers derived from flanking sequences. Sensitivity was increased by semi-nested PCR based on adjacent primers in the flanking segments. Randomly selected fragments were sequenced to confirm the specifity of PCR. The PCR detection was done at least twice for each sample and different experiments resulted in the same distribution pattern.

In cases where no proviral signals could by amplified (especially d/d minipigs; Table 1a, samples 38–52; Table

1b), the integrity of genomic DNA was tested by amplification of a ubiquitous gene, ADP ribosylation factor 3, using oligonucleotide primers derived from the human sequence (GenBank Accession No. M74491, (Tsai et al., 1991); 5′-CTTTGGAAACCTTCTCAAGAGCC-3′, 5′-TAGTGTCTCCAGAGGGGTCGAATC-3′). An additional and independent PCR analysis with LTR-specific primers (5′LTR-for, 5′-TGAAAGGATGAAAATGCAACCTAAC-3′; PBS-rev, 5′-CGCAGGATTTCCCGGCCAAC- 3′) was used to unprejudiciously amplify all proviral LTRs from genomic DNA of human cell line 293-PERV-PK. Resulting fragments were subcloned and subsequently sequenced.

Mapping of PK15 proviruses

To determine the chromosomal position of two clones isolated from cell line PK15, we used a porcine somatic cell hybrid panel (Chevalet et al., 1997), applying to this panel the PCR technique described above.

Sequence analyses

Amplification products were subcloned into pGEM-T Easy (Promega, Mannheim, Germany) and DNA sequences of both strands were determined as described previously (Niebert et al., 2002) using an ABI 377 DNA sequencing system (Applied Biosystems, Weiterstadt, Germany).

Nucleotide sequence accession numbers

The proviral sequences of Bac-PERV-A(130A12) (AJ279056), Bac-PERV-A(151B10) (AF435967), Bac-PERV-A (463H12) (AF435966), and Bac-PERV-B(192B9) (AJ279057) have been deposited in GenBank (Niebert et al., 2002) as well as those of PK15-PERV-A(58) (AJ293656) and PK15-PERV-B(213) (AJ293657) (Krach et al., 2001).

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