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High Recombination Rate of an Epstein-Barr Virus-Simian Virus 40 Hybrid Shuttle Vector in Human Cells

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The stability of an Epstein-Barr virus (EBV)-simian virus 40 (SV40) hybrid shuttle vector, the p205-GTI plasmid, was analyzed in human cells during EBV- or SV40-type replication mode. When the p205-GTI plasmid was maintained as an episomal EBV vector in the human 293 cell line, no rearrangement was detected. To induce the SV40 replication mode, cells containing the episomal p205-GTI plasmid were either transfected with vectors carrying the T antigen gene or infected with SV40. Surprisingly, we observed both production and amplification of different classes of recombinant molecules. Particular types of modifications were found in most of the recombinants. The most striking rearrangement was a duplication of the promoter and enhancer regions of SV40 which was inserted in the thymidine kinase (TK) promoter. This recombination process involved a few bases of homology, and one of the recombination junctions implicated the GC boxes which constitute the essential components of the TK and SV40 early promoters. Our results suggest that a combination of a low level of base homology and a specific DNA sequence function (promoter and enhancer sites) leads to a very high level of recombinational activity during T-antigen-dependent plasmid replication.

Shuttle vectors are used for numerous molecular studies of genomic rearrangements in mammalian cells, such as mutation and recombination. These small and easily manipulable DNA probes are suitable since they depend largely on the cellular enzymatic machinery for replication and repair. Two main classes of shuttle vectors have been designed. In appropriate host cells, simian virus 40 (SV40)-based vectors replicate with a very high efficiency, leading to a transient cell vector system (3, 29). Vectors based on bovine papillomavirus (BPV) or Epstein-Barr virus (EBV) are maintained stably as autonomous minireplicons in a variety of hosts, including, in the case of EBV, human cells (5, 7, 38). However, the copy number per cell for the EBV vectors is very low, rendering plasmid rescue in bacteria inefficient and prohibitive in routine use for mutagenesis studies. In order to improve the efficiency of plasmid rescue, it would be desirable to increase the number of EBV vector molecules per host cell. A feasible approach could be to insert the SV40 replication origin in an EBV-based shuttle vector. Switching on the SV40 replication mode by producing active SV40 T antigen in the cell could in principle generate up to 100,000 copies of the hybrid vector per permissive cell. This should greatly improve the ability to rescue the vector in bacteria. Indeed, such a system has been shown to function with BPV-SV40 hybrids (6), and an EBV-SV40 hybrid has been reported recently (14).

This paper reports the construction, replication, and stability of an EBV-SV40 hybrid replicon (p205-GTI) with either the EBV or the SV40 replication mode in human cells. Heinzel et al. (14) recently reported a similar vector for which recombination was dependent on short direct repeats inadvertently cloned in their vector. In the absence of SV40 T antigen, our vector was maintained episomally at a low copy number per cell without any detectable rearrangement. However, after introduction of T antigen, we too observed a high level of recombination but of an apparently quite different nature. During replication of our vector as an SV40-based plasmid, a high level of recombination occurred in specific sequences involving the SV40 promoter and enhancer sequences as well as the thymidine kinase (TK) promoter.

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MATERIALS AND METHODS

Plasmids. The shuttle vector p205-GTI (Fig. 1) is a derivative of plasmid p205 (34). From this vector, we conserved the origin of EBV latent replication, the EBNA-1 sequence (38), the pBR322 replication origin, and the ampicillin resistance (Amp^r) gene for selection in bacteria. To select the plasmid in human cells and in bacteria, we used the neomycin (G418-kanamycin) resistance gene, from transposon Tn*5*, under the control of two promoters: the herpes simplex virus type 1 TK promoter and a 128-base-pair (bp) fragment containing a procaryotic promoter derived from phage T7 (Set). For mutagenesis studies, we inserted, downstream of the Set promoter, the *lacZ'* gene. Finally, p205-GTI carries the 300-bp *Hin*dIII-*Hpa*II fragment of SV40 DNA containing the replication origin and the early promoters and transcriptional enhancers (21-bp and 72-bp repeats). All DNA manipulations were carried out by standard procedures (21).

The plasmids pLas (4) and pKMT11 (8) are SV40-based vectors with a defective SV40 origin. Both contain the gene encoding T antigen under the control of the SV40 early promoter (pLas) or the metal ion-inducible mouse metallothionein I promoter (pKMT11). pLas carries the ampicillin resistance gene, while pKMT11 has the kanamycin resistance gene.

Cell cultures. All DNA transfection experiments were performed by the calcium phosphate coprecipitation technique (36), and infections were done as described previously (24; C. F. M. Menck, M. James, A. Benoit, and A. Sarasin, submitted for publication). The 293-GTI line was obtained after transfection of 293 human embryonic kidney cells (11) with p205-GTI and selection in medium containing G418 (GIBCO Laboratories), 500 μg/ml during initial selection and 250 μg/ml for maintenance, as described previously (16). 293, 293-GTI, COS (10), and XP4PA-wt (4) cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum and antibiotics. For metal induction exper-

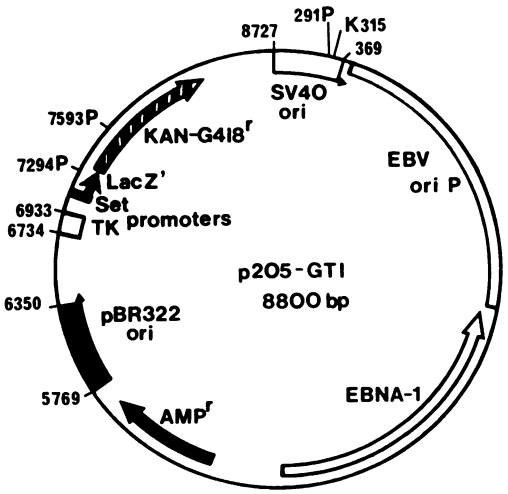

FIG. 1. Structure of p205-GTI. Open boxes (EBV and SV40 origins, EBNA-1 gene, and TK promoter) represent sequences required for replication and maintenance in human cells; solid boxes (pBR322 origin, ampicillin [AMP] resistance gene, Set promoter, and *lacZ'* gene) are bacterial portions; and the shaded box (G418 and kanamycin [KAN] resistance gene) represents sequence expressed in bacteria and in human cells. Numbering of the sequence begins within the *Bgl*I site mapped in the SV40 origin (not shown). Arrows under genes indicate transcripts. The small arrows in the SV40 and pBR322 *ori* boxes indicate the orientations of these *ori* sequences. P, *Pvu*II; K, *Kpn*I.

iments, 100 μM $ZnCl_2$ and 1 μM $CdCl_2$ were used. One day after transfection into 293-GTI cells, cells were incubated overnight with metal. Then the medium was changed, and fresh medium was added.

Plasmid recovery and DNA analysis. Extrachromosomal DNA from transfected and infected cells was purified by a small-scale alkaline lysis procedure adapted from the method described by Birnboim and Doly (2). DNA extracts were analyzed by Southern blotting and hybridization (21) with GeneScreen-Plus nylon sheets (Du Pont NEN Products) with probes, as indicated, labeled with the Multiprime labeling kit (Amersham International plc.).

Shuttling into bacteria and analysis of rescued plasmids. CsCl-purified plasmids or low-molecular-weight DNAs from cells were shuttled into *Escherichia coli* DH5α (Bethesda Research Laboratories) by the transformation method of Hanahan (13). Transformants were plated on MacConkey agar medium with ampicillin (100 μg/ml) or kanamycin (30 μg/ml). In this system, $lacZ^+$ colonies are red and *lacZ* mutants are white. Plasmid DNAs were prepared by the procedure described previously (2). Restriction analyses were performed on 0.6% agarose gels (21). Sequence analysis was done by the chain elongation termination method on double-stranded DNA templates with specific oligonucleotides.

RESULTS

The p205-GTI plasmid (Fig. 1) is an EBV shuttle vector which is episomally maintained in human cells upon selection with G418. It can be replicated in bacteria with selection by either ampicillin or kanamycin or both. In addition, it contains the SV40 replication origin, which is activated in the presence of SV40 T antigen. Therefore, this vector can present two different modes of DNA replication and amplification.

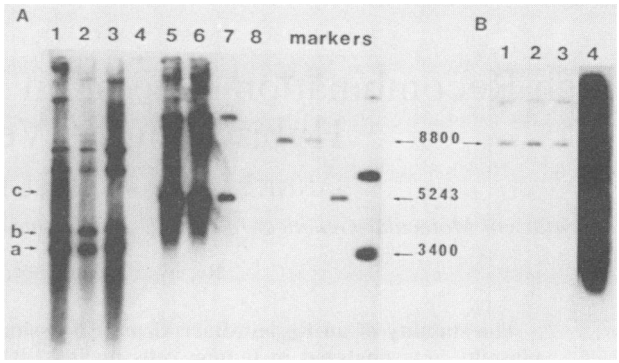

FIG. 2. Agarose gel blot hybridization analysis of low-molecular-weight DNAs prepared from 293-GTI cells. DNA isolated from 10^6 cells was loaded for all lanes, and the filters were hybridized with the radiolabeled *Pvu*II fragment (nucleotides 7593 to 291) of p205-GTI as the probe, spanning the SV40 replication origin sequences. (A) Three days after T-antigen boost: transfection with pLas (lane 1) or pKMT11 in the absence (lane 2) or the presence (lane 3) of metal; SV40 infection at three dilutions of a 10^8-PFU/ml stock of virus, 1:40 (lane 5), 1:100 (lane 6), and 1:1,000 (lane 7). Lanes 4 and 8 correspond to plasmid p205-GTI DNA (not visible at this exposure) recovered from mock-transfected or mock-infected cells, respectively. Marker lanes, 1 and 0.3 ng of p205-GTI; 1 ng of form I SV40; 1 ng of forms I and II 3,400-bp plasmid markers. Arrows (a, b, c) indicate form I of the main recombinants obtained after T-antigen boost by transfection (arrows a and b) and by infection (arrow c). Higher bands represent form II of the main recombinants. (B) Lanes 1, 2, and 3 correspond to three different extractions of p205-GTI from 293-GTI cells without the T-antigen boost. Lane 4 shows, at the same exposure, the recombinant plasmids produced 3 days after T-antigen boost by transfection with pKMT11, identical to that in lane 3 in panel A.

To study the genetic stability of the vector, it was transfected into adenovirus-transformed human embryonic 293 cells. After G418 selection, 293-GTI lines were obtained and cultured for at least 2 months. In these cells, the vector was replicating as a stable episome (Fig. 2B). The copy number per cell was estimated to be between 1 and 10. To introduce T antigen into these cells, two strategies were used: transfection of plasmids carrying the T-antigen sequence or infection by wild-type SV40 virus. The plasmids used for transfection, pLas and pKMT11, were nonreplicating SV40-based vectors. The T-antigen gene was under the control of two different promoters: the SV40 early promoter (pLas) and the metal ion-inducible metallothionein I promoter (pKMT11). Three dilutions were used for the infecting SV40 virus: 1:40, 1:100, and 1:1,000 at 10^8 PFU/ml. These different ways of introducing the SV40 T-antigen gene would be expected to give various levels of T-antigen expression. According to Heinzel et al. (14), we refer to this strategy as a T-antigen boost.

293-GTI cells were either infected with SV40 or transfected with pLas or pKMT11. One day after transfection, the 293-GTI cells containing pKMT11 were incubated, or not, overnight in the presence of heavy-metal ions. Extrachromosomal DNA was recovered from cells 3, 5, 10, or 15 days after transfection or infection and analyzed by Southern blotting (Fig. 2A). Expression of the T-antigen gene in the 293-GTI cells did not lead to significant p205-GTI amplification but yielded new plasmid species. The same plasmid pattern was found at 3, 5, 10, and 15 days after SV40 infection or after transfection of pLas and pKMT11, with or without metal induction. After a T-antigen boost by trans-

TABLE 1. Sensitivity to antibiotics of bacteria transformed by rescued shuttle vectors[a]

Transfecting plasmid or infecting virus strain	No. of colonies		Ratio, Kan^r/Amp^r colonies
	Kan^r	Amp^r	
T-antigen boost			
Transfection with:			
pLas	708	2	354
pKMT11, no addition	2,061	17	121
pKMT11 plus $ZnCl_2$-$CdCl_2$	3,730	15	249
Infection with SV40	80	2	40
Transfection with p205-GTI[b]	180	183	1

[a] Shuttle vectors from 293-GTI cells were submitted to T-antigen boost by transfection or infection (1:40 dilution of 10^8 PFU/ml) were transfected in bacteria, and kanamycin and ampicillin-resistant colonies were scored on MacConkey agar. Data from plasmids rescued after 3 and 5 days have been pooled.

[b] $CsCl_2$-purified p205-GTI.

fection (Fig. 2A, lanes 1, 2, and 3), two predominant classes of recombinant plasmids were distinguished with approximate sizes of 3,300 and 3,900 bp (Fig. 2A, arrows a and b). These two bands hybridized with SV40 and pBR322 replication origin probes but not with a probe corresponding to the EBV origin of replication. After SV40 infection (Fig. 2A, lanes 5, 6, and 7), the quantity of amplified recombinant plasmids was lower (compared with that after transfection), but they were present at all virus dilutions. The main recombinant molecules (Fig. 2A, arrow c) comigrated with SV40 DNA and also hybridized with SV40 and pBR322 origins but not with the EBV origin.

To analyze p205-GTI stability and the recombinant structures, DNA preparations from mammalian cells were transfected into bacteria. After selection with ampicillin, a few colonies were obtained, while selection with kanamycin produced many more colonies (Table 1). Similar results were found with both induction methods and at all cell extraction times. The ratio of colonies that were resistant to kanamycin to those resistant to ampicillin is shown in Table 1. When purified p205-GTI was transfected directly into bacteria, the same number of colonies were found on kanamycin and ampicillin plates. From Table 1, one can deduce that the amplified recombinants which have conserved an active pBR322 replication origin carry the kanamycin resistance gene but almost all exhibit deletions in the ampicillin resistance gene. The *lacZ'* gene was conserved intact, since colonies appeared red on MacConkey agar.

A large number of plasmids rescued from cells after three independent transfection experiments (T-antigen boost with pLas or pKMT11 with or without metal induction) or after two different SV40 infections (1:40 and 1:100 dilutions) were analyzed on agarose gels. The vectors isolated from the few ampicillin-resistant colonies appeared to be identical to the p205-GTI, while plasmids from kanamycin-resistant colonies were heterogeneous. The sizes of the rescued plasmids are shown in Fig. 3. A large range of sizes from 12,000 to 3,000 bp was observed, but as previously seen by Southern blotting (Fig. 2A), predominant recombinant classes of 3,300 and 3,900 bp appeared after T-antigen boost by transfection (Fig. 3A). After SV40 infection (Fig. 3B), the size of recombinant molecules was also heterogeneous, with the majority being ca. 6,000 bp. To determine the recombinant structures, rescued plasmids were digested with various restriction enzymes. Representative results are presented for the enzyme *Pvu*II in Fig. 4. Most of these plasmids showed more

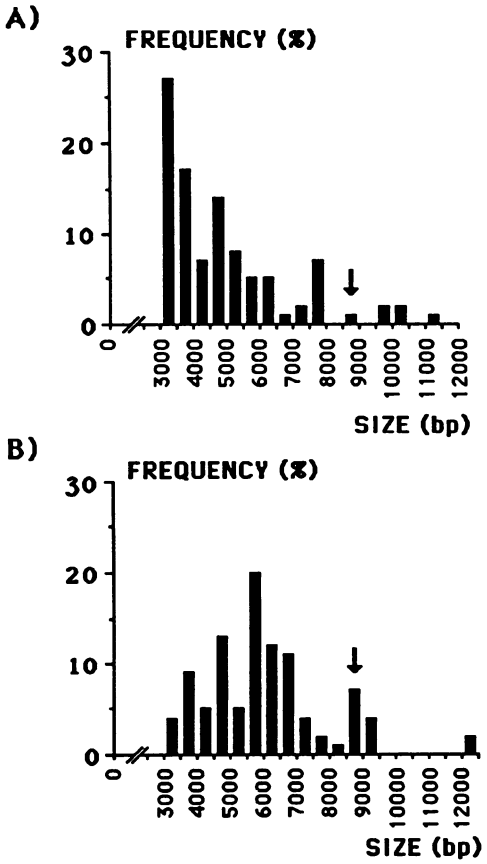

FIG. 3. Size distribution of recombinant plasmids. DNA from human cells recovered after the T-antigen boost by transfection (A) or by infection (B) were used to transfect bacteria. DNA from 100 kanamycin-resistant colonies was analyzed on agarose gels. Their sizes were compared with those of three (undigested) marker plasmids and estimated after digestion by different restriction enzymes (*Pvu*II, *Kpn*I, and *Pvu*II-*Bgl*I). Arrows indicate p205-GTI.

*Pvu*II sites than the original p205-GTI (three sites, shown in Fig. 1). In particular, a new 700-bp fragment (Fig. 4, arrow) was often found in the recombinant plasmids, although they derived from independent transfection and infection experiments. The 3,300-bp plasmids produced after T-antigen gene transfection fell into two types based on their restriction

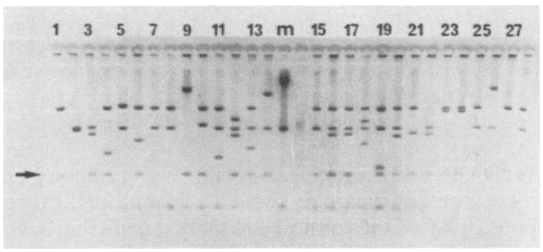

FIG. 4. *Pvu*II restriction pattern of recombinant plasmids obtained after five independent T-antigen boost experiments by transfection (lanes 1 to 18) or by infection (lanes 19 to 28). The restriction digests of the pR1, pR2, and pR3 plasmids are shown in lanes 1, 2, and 3, respectively. Lanes 15 to 18 are digests of the three largest recombinants. Lane m, *Pvu*II digestion of the marker plasmid, p205-GTI, which produced three fragments of 7,003, 1,498, and 299 bp. Arrow denotes the position of the new 700-bp fragment present in most recombinants.

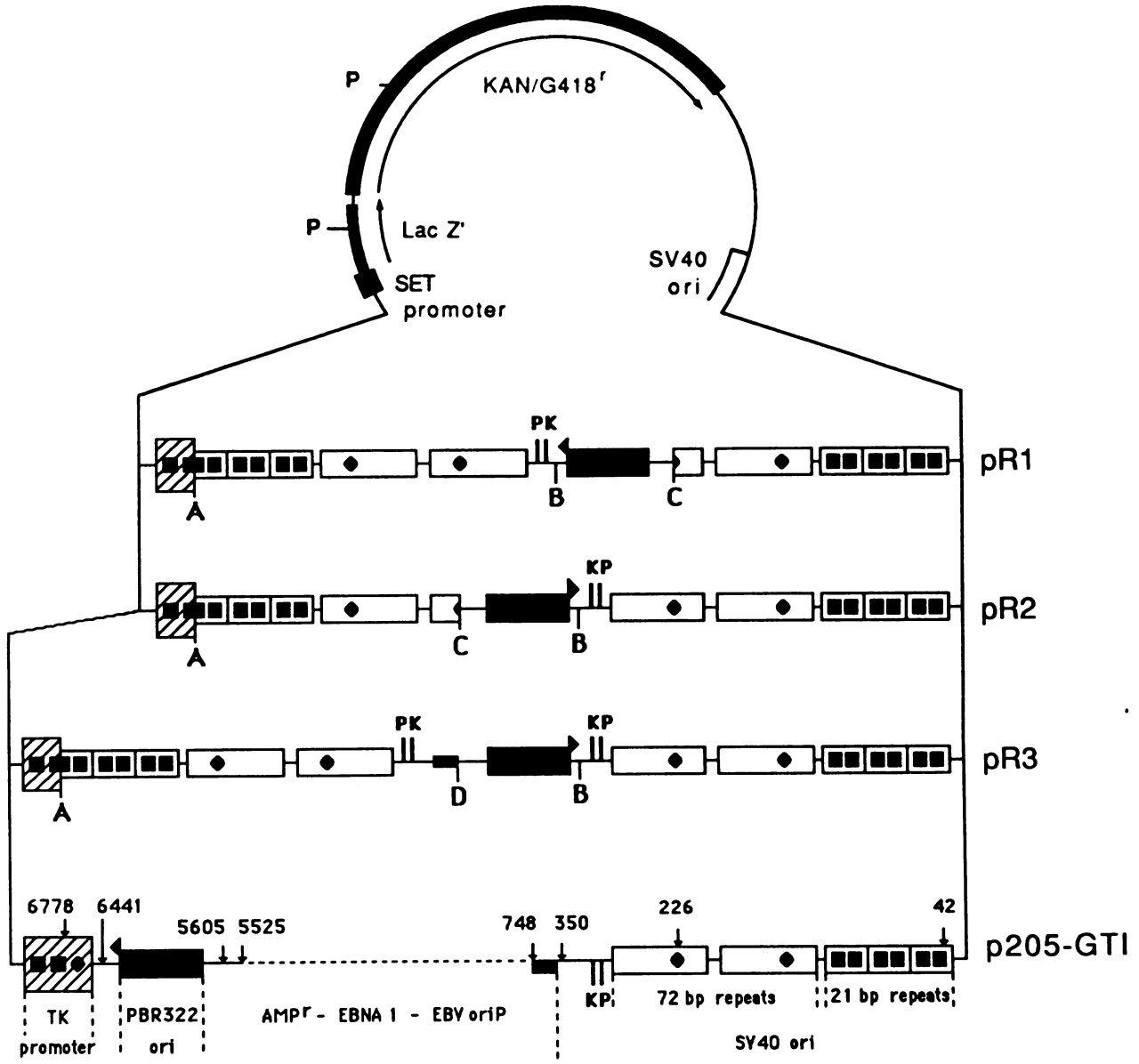

FIG. 5. Schematic structures of the three predominant recombinant plasmids, pR1 (3,300 bp), pR2 (3,300 bp), pR3 (3,900 bp), and p205-GTI (8,800 bp). The maps are based on data from restriction analysis and sequencing of the recombination junctions (Fig. 6). Numbering is the same as in the p205-GTI map (Fig. 1); numbers on the p205-GTI section denote the crossover points found in the different recombinants. Orientation of the pBR322 origin is indicated by the arrowhead at one corner of the solid black box. The small black box shown between positions 350 and 748 is the section of the *oriP* fragment which is involved in junction D (pR3). See Fig. 1 for a description of the circular section of the map. Figure is not drawn to scale. Symbols: ■, GC box; ◆, octamer; P, *Pvu*II; K, *Kpn*I; A, B, C, and D, recombination junctions shown in detail in Fig. 6.

patterns. Digests of these are shown in Fig. 4, lanes 1 and 2. These two types of plasmids (called pR1 and pR2) together represented 30% of the total plasmids rescued (Fig. 3A). We also used the *Kpn*I restriction enzyme, which cuts p205-GTI once near the SV40 enhancers (Fig. 1). The *Kpn*I restriction pattern revealed that the smallest recombinants contained one or two sites, while the largest recombinants contained three *Kpn*I sites (not shown).

To characterize the recombination sites, we restriction mapped and then sequenced the junction regions of recombinants corresponding to each of the three predominant classes: pR1 (3,300 bp), pR2 (3,300 bp), and pR3 (3,900 bp). Their maps (Fig. 5) revealed that the three recombinants were very similar. The common features of their structures are as follows: (i) they had lost the EBV replication origin, the EBNA-1 gene, and the ampicillin resistance sequence; (ii) they carried duplications of the SV40 early promoter and enhancer sequences, removing the 5′ extremity of the TK promoter (including the octamer sequence [27]; see Fig. 5); and (iii) the pBR322 origin sequence was conserved but was included between the original and duplicated SV40 promoter elements.

Sequences of recombination junctions, determined for independent recombinant plasmids rescued from bacteria,

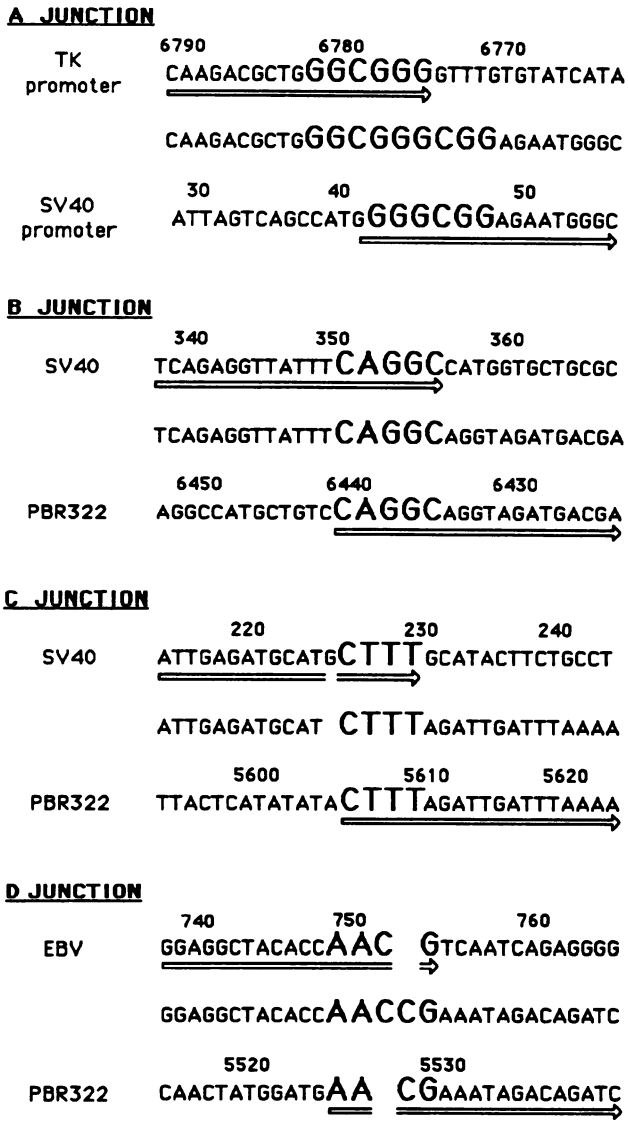

FIG. 6. Sequences of the recombination junctions whose positions are shown in Fig. 5. The nucleotide sequences of the parental DNAs are given above and below the recombinant sequence for each junction. Nucleotides in boldface type are common to both parents involved in the recombination junctions. The sequences underlined with arrows were recovered in the recombinants. Numbers refer to the p205-GTI map (Fig. 1 and 5).

are shown in Fig. 6. Several points emerged from these results. (i) Although each recombinant contained at least three recombination junctions, we only found four different kinds of recombination sites (Fig. 5). Indeed, junctions A and B were identical in all three recombinants, junction C was common to two recombinants (pR1 and pR2), and junction D was only found in pR3. (ii) In all four junctions, the crossover point occurred within parental DNA sequences which presented 4, 5, or 6 bases of homology. (iii) In junction A, the rearrangement event was the result of recombination between the first GC box (nucleotides 42 to 47) of the SV40 early promoter (1) and the distal signal II (nucleotides 6777 to 6782) of the TK promoter (22), which is also a GC box. (iv) The SV40 sequences involved in junction C were within the octamer defined as the enhancer core of the 72-bp SV40 repeat (30). (v) The pR1 and pR2 maps showed (Fig. 5 and 6) that the difference between these two plasmids was due to the inversion of the pBR322 origin and the two SV40 sequences, which together present 161 bp of homology.

To test whether the three main classes of recombinants could replicate in mammalian cells, we transfected them into monkey COS and human XP4PA-wt cells, both of which constitutively express SV40 T antigen (4, 10). Low-molecular-weight DNAs were extracted, digested with *Dpn*I, and analyzed by Southern blotting or used to transfect bacteria. The three recombinants were replicated by the two cell lines and therefore had a functional SV40 replication origin (not shown). p205-GTI was also transfected into these cells. No amplification was detected, although these cells were able to replicate SV40-based shuttle vectors (4, 10, 32), and we have observed that the recombinants replicated better than p205-GTI in COS and XP4PA-wt cells (data not shown).

DISCUSSION

We have constructed a shuttle vector containing both the SV40 and EBV replication origins, p205-GTI (Fig. 1), and shown that it replicated as an EBV vector in human cells in the absence of T antigen (Fig. 2B). After the initiation of the SV40 replication mode by T-antigen boost, we observed the rapid appearance of many recombinant species. No amplification of the hybrid p205-GTI was observed. The major species detected by Southern blotting (Fig. 2A) appeared to correspond to the major species found after shuttling into *E. coli* (Fig. 3). Restriction analysis (Fig. 4) and DNA sequencing (Fig. 6) allowed us to determine the maps of three predominant types of recombinants (Fig. 5). Their essential characteristics are as follows. (i) They are derived forms of the p205-GTI plasmid and do not contain sequences from cellular DNA. Similarly, they do not contain sequences from the infecting or transfecting vectors containing the T-antigen information, since these vectors induce the same recombinants, although their own DNA sequences are different except for the T-antigen gene. Therefore, all sequences involved in the rearrangements arise from the p205-GTI plasmid. (ii) These recombinants showed an inverted duplication of the SV40 promoter and enhancer sequences. One extremity of the duplication insertion involved two GC boxes, the first one arising from the first SV40 21-bp repeat (1) and the other one from the distal signal II of the TK promoter (22). (iii) Although the pBR322 replication origin presumably is not required for amplification in human cells, the predominant recombinants conserved this sequence, but it was rearranged between the normal and the duplicated SV40 promoter sequences. We cannot exclude that the main bands revealed by Southern blotting also contained plasmids without the pBR322 replication origin. However, precise analysis of the types of rearrangements carried out in the human cells can only be performed on individual recombinants rescued in bacteria. Restriction analysis (Fig. 4) showed that the predominant and the other recombinants isolated after five independent experiments of T-antigen boost by transfection or infection presented common fragments, some of which differed from those found with p205-GTI. Particularly, a new *Pvu*II fragment (700 bp) was generated by the duplication and the recombination between SV40 and TK promoters, as shown in the pR1 and pR3 maps. Since this fragment was present in most of the recombinants, the general recombination pattern and the sequences involved in this duplication process were certainly similar for most of the recombinants.

With regard to the mechanism of recombination, our experiments do not allow us to comment in detail. This is further complicated by the multiple recombinations that each recombinant has undergone. For example, it is not clear to what extent homology is required for the events we have observed. While all recombination junctions have 4 to 6 bases homologous to the two parental recombining DNAs, it has been estimated that the minimal amount of homology required for homologous recombination in mammalian cells is 14 bp (31). It is plausible that nonhomologous events may arise randomly throughout the p205-GTI genome, followed by a selective advantage for certain recombinants. However, Heinzel et al. (14), using a similar system with an EBV-SV40 hybrid shuttle vector, did not detect any significant nonhomologous recombination during the amplification of their vectors. Therefore, random rearrangements followed by genetic selection are not sufficient to explain the production of the recombinants. We did not detect any simple deletion intermediates produced by random recombination. Indeed, the large recombinants (ca. 8,000 bp) we analyzed presented a number of *Pvu*II sites (Fig. 4, lanes 15 to 18) and *Kpn*I sites (not shown) greater than those of the original p205-GTI, indicating that they derived from multiple rearrangement events in the SV40 enhancer sequences rather than via random recombination of the original vector. On the other hand, we can postulate that some sequences of p205-GTI could be specific sites for recombination. One of these sites could correspond to the SV40 sequences spanning the enhancer and promoter regions. Indeed, recombinant SV40 viruses have already been reported (19, 20), which frequently corresponded to multiple copies of identical SV40 sequences derived from the replication origin. This feature was interpreted to arise as a consequence of a strong selective advantage of the recombinant due to enhancement of replication. In the case of the p205-GTI recombinants, the SV40 replication origin was not duplicated, but it is striking that all the SV40 sequences involved in recombination junctions have been identified previously as sequences implicated in the crossover points leading to SV40 variants (17, 26, 37) or SV40-adeno-associated virus recombinants (12). These recombination hot spots share short common sequences with the TK promoter (Fig. 6, junction A) and the pBR322 sequence (Fig. 6, junctions B and C) present in p205-GTI.

To summarize our conclusions so far, homology per se would not seem to be sufficient to account for the high level and specific nature of the recombinants we observed. We tentatively propose that the SV40 and TK promoter regions could predispose to recombination due to the more open configuration in transcriptional complexes or to the secondary structure of the DNA near the crossover points (9, 18, 25). The GC boxes involved in the recombination junctions are recognized, in a sequence-specific manner, by the cellular transcription factor, the protein Sp1 (23). Current structural studies of transcription factors and upstream promoter elements have been combined into a model in which each transcription factor protein interacts with several DNA-binding sites or, alternatively, a protein dimer of the transcription factor interacts with two separate DNA-binding sites, causing a looping-out of intervening DNA (28). Thus, in our case it is easy to visualize how such transcription factor-mediated binding could bring the TK and SV40 GC boxes into proximity, which in turn may greatly increase recombination based on the weak homology between these sites. Such recombination may be synergistically enhanced by concomitant changes in the secondary structure induced by binding of the transcription factors or the structure intrinsic to these sequences (15, 28). Recently published data offer some support for this speculation. There has been identified in *Saccharomyces cerevisiae* (35) a DNA sequence (*HOT1*) which stimulates genetic exchange and corresponds to sequences regulating transcription by RNA polymerase I; the authors postulated that "transcriptional activity may be a general feature controlling the frequency and distribution of recombination events in eukaryotic cells" (35). Therefore, it is possible, in the case of the p205-GTI, that both features, sequence homology and transcriptional activity, play a role in the apparent site-specific recombination that we observed.

The absence of detectable amplification of the original vector could indicate that the recombinational events take place very early once the SV40 replication mode is turned on. The role of the SV40 T antigen is not clear, since it has at least two different effects on the p205-GTI vectors. First, it binds to the SV40 promoter sequences for promoting transcriptional activity, which may lead by itself to recombinational events, as discussed previously. Second, it promotes efficient vector replication in an SV40 mode. It was demonstrated (33) that, in a transient-expression assay, neither SV40 T antigen nor replication is essential for nonhomologous recombination in mammalian cells. It is not clear whether those results (33) are pertinent to our system, as it is well known that transfected DNA is subjected to more or less random breakage and reunion.

Since Heinzel et al. (14), with a similar system, have obtained amplication of their EBV-SV40 shuttle vector, the absence of detectable amplification of p205-GTI and the generation of recombinants seem to indicate that these phenomena are due to the particular structure of p205-GTI. By comparison between the plasmid of Heinzel et al. and the p205-GTI vector, we note that the SV40 replication origins are in opposite orientation with regard to the TK promoter and that their plasmid carries only a truncated region of the 72-bp repeat. To correlate our results with those of Heinzel et al. (14) and to attempt to test our hypothesis about the mechanism and involvement of transcriptionally active sequences in recombination, we are currently performing the same type of experiments (T-antigen boost) with a new series of vectors with specific deletions and orientations of these elements.

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