Activation and Targeting of Immunoglobulin Switch Recombination by Activities Induced by EBV Infection[1]

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EBV is strongly associated with Burkitt's lymphoma, a B cell malignancy. In certain types of Burkitt's lymphoma, the c-*myc* gene has undergone translocation to the S regions associated with heavy chain switch recombination. It has not been established whether EBV infection induces recombination activities, which in turn promote translocation of c-*myc,* or whether translocation precedes viral infection and provides a growth advantage that is further enhanced by factors encoded or induced by the virus. To distinguish between these possibilities, we have compared the level of switch recombination activities in the EBV-negative lymphoma, BJAB, and in its EBV-infected derivative, BJAB-B1, in experiments that assayed recombination of an extrachromosomal switch substrate during transient transfection. We have found that BJAB-B1 and other EBV-positive B cell lines supported high levels of recombination of switch substrates, to produce junctions like those found in products of chromosomal switch recombination. In contrast, BJAB did not support comparable levels of switch substrate recombination. In EBV-positive B cell lines, the ability to support switch substrate recombination correlated with levels of LR1, a B cell-specific factor which is a transcriptional regulator of c-*myc* and which also appears to function in switch recombination. Our observations support the hypothesis that EBV infection can induce activities that affect switch recombination and thus contribute to the translocations of c-*myc* to the S regions that characterize certain classes of lymphomas. ***The Journal of Immunology,* 1999, 163: 6659–6664.**

Epstein-Barr virus has been implicated in the pathogenesis of many benign and malignant lymphoproliferative disorders in humans (1, 2). The most common malignancy associated with EBV is Burkitt's lymphoma (BL),[4] a tumor characterized by translocations of the c-*myc* oncogene from its normal position on chromosome 8 to the Ig heavy and light chain loci. In endemic BL, translocation typically involves regions that contain recombination signal sequences that participate in V(D)J joining, which creates the Ag receptor. In sporadic BL, the c-*myc* gene typically has undergone translocation to the Ig heavy chain switch (S) regions, which are involved in switch recombination. In vitro, EBV establishes latent infection in B cells that have undergone V(D)J recombination and stimulates these cells to proliferate and express markers characteristic of cell activation. This has raised the question of whether EBV infection might activate switch recombination activities, which in turn are responsible for c-*myc* translocation in sporadic BLs. The possibility that switch recombination activities might be induced by EBV is of particular interest because the EBV genome contains a region that bears striking similarities to the Ig heavy chain switch regions. This region, referred to as the BamW repeats, consists of 9 to 11 tandem repeats of a G-rich 3-kb segment and is frequently involved in EBV genomic rearrangements (3, 4).

Our laboratory has developed a transient transfection assay for the presence of activities that support switch recombination (5–9). This assay measures recombination between Sμ and Sγ3 region sequences carried by a shuttle vector. In the first step, recombination occurs during transfection of mammalian cells; in the second step, recombinants are enumerated by genetic selection in *Escherichia coli*. Similar substrates have been developed by Lieber and collaborators (10, 11). Other laboratories have developed integrating substrates that undergo recombination in transfected cell lines, including substrates carried on both plasmid and retroviral vectors (12–15).

The extrachromosomal switch substrates have proved to be a powerful tool for assaying switch recombination activities in murine cells. The substrates effectively recapitulate chromosomal switch recombination, in that they recombine at high frequencies in primary LPS-activated murine B cells (5) and in pre-B and B cell lines, but not other cell types (6, 9, 10); the majority of recombination junctions map to sites within the S regions (5–11); junctions are heterogeneous in site and sequence (5–11); and mutations are evident within a few hundred nucleotides of one side of the junction (11). However, thus far the use of extrachromosomal switch substrates has been limited to murine cell lines, because the substrates developed by both our own laboratory (5–9) and Lieber's laboratory (10, 11) are carried on a polyoma-based shuttle vector, and polyoma does not replicate in human cells. To study how EBV infection affects switch recombination activities, we have now modified the extrachromosomal substrates to allow analysis of recombination in human cells. Using recombination cassettes carried on an SV40-based shuttle vector, we have examined recombination activities in the EBV-negative lymphoma, BJAB, and its EBV-positive derivative, BJAB-BI, as well as in other cell types. We have found that recombination levels were very low in BJAB but that BJAB-B1 and group I and group III EBV-positive BL cells supported high levels of switch substrate recombination. EBV infection can therefore induce activities that support switch

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[4] Abbreviation used in this paper: BL, Burkitt's lymphoma; EBNA, Epstein-Barr nuclear Ag; LMPs, latent membrane proteins.

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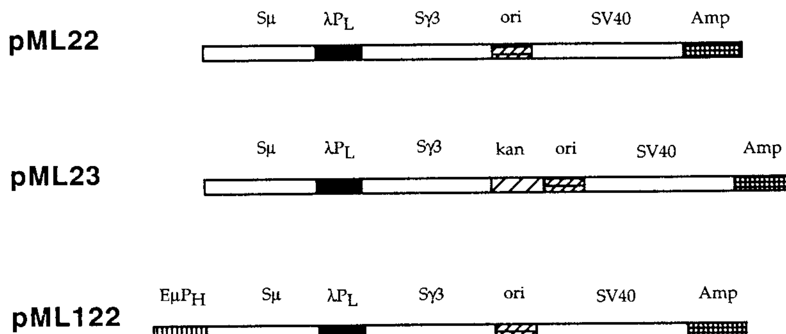

FIGURE 1. Extrachromosomal switch substrates. All constructs carry Sμ and Sγ3 switch region sequences flanking the leftward promotor of phage λ (λP_L). These sequences are cloned into a shuttle vector containing the SV40 origin and large T Ag to support replication in human cells; and a bacterial origin (*ori*) and ampicillin resistance gene (*Amp*). In pML23, a kanamycin resistance gene (*Kan*) is cloned downstream of the Sγ3 region. In pML122, a fragment containing the Ig heavy chain intron enhancer and promoter, EμP_H, is inserted just upstream of the Sμ region.

recombination. One B cell-specific activity implicated in switch recombination is LR1, a sequence-specific duplex DNA binding factor identified and characterized by our laboratory (16–18). LR1 is a transcriptional regulator of the c-*myc* protooncogene (19) and of the EBV Fp promotor (20), and LR1 binds specifically to the G-rich consensus sequence, GGNCNAG(G/C)CTG(G/A). We further show that recombination frequencies correlated with levels of LR1 duplex DNA binding activity.

Our data support a model in which EBV infection mimics Ag activation by inducing activities essential to switch recombination. These activities may be important in replication or recombination of the EBV genome, and they are also implicated in the translocations of c-*myc* to the Ig loci.

Materials and Methods

Construction of switch substrates

The extrachromosomal switch substrates were derived from pHL22 and pHL122 (5), which were designed to assay recombination in murine cells and carried the polyoma early region. To allow replication in human cells, we replaced the polyoma early region with the SV40 large T and replication origin to create the following constructs:

pML22. The recombination cassette was excised from pHL22 as a 5.9-kb *Not*I-*Eco*RV fragment, which includes 2.2 kb of Sμ sequence, the leftward promoter of bacteriophage λ (λP_L), and 2.5 kb of Sγ3 sequence. This fragment was cloned into shuttle vector pGG51 (a gift from Dr. Michael Lieber, University of Southern California, Los Angeles, CA), which contains the SV40 origin of replication and large T Ag coding sequence, the prokaryotic ColE1 replication origin, and an ampicillin resistance gene; pGG51 had been digested with *Sal*I and *Bgl*II and adapted with *Not*I linkers, as had the vector (Fig. 1).

pML23. A 962-bp *Hin*dIII fragment carrying a kanamycin resistance gene was excised from pBS.Km2, filled with Klenow, and inserted into the *Nar*I site of pML22 just downstream of Sγ3 (Fig. 1).

pML122. A 6.8-kb *Xho*I-*Eco*RV fragment containing the Ig heavy chain intron enhancer and promoter (EμP_H), Sμ, λP_L, and Sγ3 sequences was excised from pHL122 and cloned into pGG51 at its *Sal*I and (filled) *Bgl*II sites.

The λP_L promoter is a conditionally lethal marker. Plasmids carrying λP_L cannot produce antibiotic-resistant transformants of nonlysogenic strains of *E. coli*, because active transcription of λP_L interferes with plasmid replication. These plasmids can replicate in λ lysogens, in which the prophage produces the λ repressor protein which prevents transcription of λP_L. Upon recombination involving the S regions, λP_L is deleted, and the resulting recombinants can transform a nonlysogenic strain of *E. coli*.

Plasmids were propagated in *E. coli* DH10B(λ) and purified by Qiagen (Chatsworth, CA). Before transfection of eukaryotic cells, all plasmid preparations were tested for the λP_L marker by comparison of the number of transformants of DH10B and the isogenic lysogen, DH10B(λ). The frequency of inactivation of λP_L was in all cases $<10^{-4}$, which is in the range of the mutation rate in *E. coli* (data not shown). Restriction mapping of plasmid recovered from DH10B, in which λP_L had been inactivated, showed that the mutation events that had occurred during propagation in *E. coli* were single base changes or deletions and insertions so small that they did not affect the restriction map.

Cell lines, cell culture, and transfection

The following human cell lines were used in the switch substrate recombination assay: Raji, a group III EBV-positive BL (21); Akata, a group I EBV-positive BL (22); BJAB, an EBV-negative BL (23); BJAB-B1, an EBV-infected derivative of BJAB (24); BJE2, an Epstein-Barr nuclear Ag (EBNA)-2 cDNA-transfected derivative of BJAB (25); and HT1080, a fibrosarcoma cell line (26). B cell lines were cultured in RPMI 1640 and HT1080 was cultured in MEM-α. All media were supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-ME.

Optimal transfection conditions were determined by transfection with a reporter construct in which a chloramphenicol acetyltransferase (*CAT*) gene was driven by the CMV IE1 promoter-enhancer (20). Akata, BJAB-B1, BJAB, and BJE2 cell lines were transfected using DEAE-dextran, as described previously (9). Briefly, 2×10^7 cells were pelleted, washed with RPMI 1640, and resuspended in 1 ml RPMI 1640 containing 500 μg/ml DEAE-dextran (Pharmacia, Piscataway, NJ) and 10 μg Qiagen-purified plasmid DNA. After 30 min incubation at 37°C, cells were washed with RPMI 1640 and resuspended in 30 ml culture medium. For transfection of Raji cells, 4×10^7 cells were washed with PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4), resuspended in 0.8 ml PBS, and mixed with 10 μg DNA. The reaction was loaded into a 0.4-cm gap electroporation cuvette (Bio-Rad, Foster City, CA) and electroporated at 350 V, 960 μF on a Bio-Rad gene pulser. Transfection of HT1080 cells was conducted with the Calcium Phosphate Transfection System (Life Technologies, Gaithersburg, MD) as recommended by the manufacturer.

Switch substrate recombination assay

The switch substrate recombination assay has been described in detail previously (5, 6, 9). Briefly, cells were transfected with substrate, and 42 h after transfection low m.w. DNA was isolated by rapid alkaline lysis. A compatible plasmid pACYC184, which contains a gene encoding chloramphenicol resistance, was added to recovered DNA as an internal control for transformation, and samples were digested with *Dpn*I to destroy molecules that had not replicated in the eukaryotic cells. Recombination substrates were propagated on dam^+ *E. coli* strains, whereas pACYC184 was propagated on a dam^- strain of *E. coli* and was therefore resistant to *Dpn*I digestion. Equal aliquots of DNA were transformed into *E. coli* DH10B and the isogenic λ lysogen DH10B(λ) by electroporation. The recombination frequency (*R*) was calculated as the ratio of ampicillin-resistant (Amp^R) (or in some experiments kanamycin-resistant (Kan^R)) transformants of the nonlysogen compared with the lysogen, normalized for transformation efficiency as measured by chloramphenicol-resistant (Cm^R) transformants:

$$R = \frac{Amp^R \text{ transformants of DH10B}}{Amp^R \text{ transformants of DH10B}(\lambda)} \times \frac{Cm^R \text{ transformants of DH10B}(\lambda)}{Cm^R \text{ transformants of DH10B}}$$

Mapping of recombinants

Minipreps of recombinant DNA were produced by alkaline lysis, and deletion endpoints of recombinant plasmids were mapped by digestion with *Hin*dIII, *Not*I, and *Xho*I.

Gel mobility shift assay

Gel mobility shift assays were conducted as described (16, 19). Nuclear extracts from cultured cells were incubated with ^{32}P-labeled synthetic duplex oligonucleotides containing an LR1 site from the murine Sγ1 switch region, or a mutant site (G14A) differing at a single base pair. DNA-protein complexes were resolved in a 7% polyacrylamide gel in 90 mM Tris-borate, 1 mM EDTA, pH 8.3.

Table I. *Recombination frequencies of pML22*[a]

	Cell Lines	DH10B		DH10B(λ)			
		Amp^R	Cm^R	Amp^R	Cm^R	*R* (%)	*R* (avg.)
EBV^-	BJAB	1	346	170	336	0.6	
		4	408	159	248	1.5	
		3	501	214	382	1.1	1.1 ± 0.3%
EBV^+	BJAB-B1	62	82	7200	442	4.6	
		14	58	1920	472	5.9	
		20	681	461	745	4.7	
		27	317	381	296	6.6	5.5 ± 0.5%
EBV^+ BL (group I)	Akata	18	111	3600	1644	7.4	
		11	42	2660	530	5.2	
		33	115	2209	808	10.5	
		36	47	5874	358	4.7	
		17	97	2187	583	4.7	6.5 ± 1.1%
EBV^+ BL (group III)	Raji	13	35	2112	403	7.1	
		20	43	1614	390	11.2	
		21	140	3560	746	3.1	
		31	45	1608	256	11.0	
		14	39	426	310	26.2	11.7 ± 3.9%
$EBNA2^+$	BJE2	9	719	807	1395	1.9	
		11	712	815	646	1.2	
		403	1425	8240	700	2.4	
		225	1472	12600	1644	2.0	1.9 ± 0.2
Fibroblast	HT1080	19	94	3658	282	1.6	
		13	1474	3120	2328	0.7	
		5	515	1200	980	0.8	1.0 ± 0.3%

[a] pML22 was transiently transfected into the cell lines shown above. After 42 h, low m.w. DNA was recovered, *Dpn*I digested, and assayed for recombination. The table shows the number of Amp^R and Cm^R colonies recovered after transformation of DH10B and the isogenic lysogen, DH10B(λ). Each line represents data from a single transfection experiment. The recombination frequency (*R*) was calculated as the ratio of Amp^R transformants in DH10B to those in DH10B(λ), normalized by the control Cm^R transformants. The rightmost column shows the average recombination frequency (*R* (avg.)) ± SE.

Results

Recombination of switch substrates is elevated in human EBV⁺ BL cell lines

We first assayed recombination of pML22 in BJAB, its EBV-infected derivative, BJAB-B1, and in the human fibroblast line, HT1080. As shown in Table I, the recombination frequency in BJAB was $R = 1.1\%$. This low level was comparable with that in the fibroblast line, HT1080. In contrast, the recombination frequency in BJAB-B1 was 5-fold higher, $R = 5.5\%$. This is comparable with the level observed with murine substrates recovered from transfection of B cell lines (9).

EBV carries ~100 genes, but cells transformed by EBV express only a small fraction of them. BLs are classified as group III if they express EBV genes including the nuclear proteins, EBNA-1, -2, -3A, -3B, -3C, and -LP, several latent membrane proteins (LMPs), and two EBER RNAs; and as group I if the only EBNA expressed is EBNA-1. We tested the ability of representatives of these two classes of EBV-transformed BLs to support switch substrate recombination. The recombination level in Akata (group I) was $R = 6.5\%$, comparable with that of BJAB-B1. It was significantly higher in Raji, $R = 11.7\%$. BJE2 cells, which stably express EBNA-2 but no other EBV genes, supported only background levels of recombination. These results show that genes encoded by EBV enhance switch substrate recombination in these BL cell lines.

Enhanced recombination activities in EBV⁺ lymphoma cells target switch region sequences

It was critical to map recombinants to determine whether recombination was targeted to S region sequences. In the construct pML22, the ampicillin resistance marker is directly upstream of Sμ, and the bacterial replication origin is directly downstream of Sγ3. This biases recovery of molecules in which recombination breakpoints are beyond the S regions. To map recombination junctions, we therefore constructed another switch substrate, pML23, in which a 1-kb kanamycin resistance gene was inserted just downstream of the Sγ3 region. After transfection of human cells with pML23, we selected either ampicillin-resistant or kanamycin-resistant recombinants. The ampicillin-resistant recombinants (Amp^R) included all those with acceptor endpoints downstream of the Sγ3 region, whereas the kanamycin-resistant recombinants (Kan^R) included all those with donor endpoints located upstream of the Sμ region. We compared recombination frequencies of pML22 and pML23 to determine whether introduction of the kanamycin cassette affected recombination levels. As shown in Fig. 2, recombination frequencies of the two constructs were comparable, regardless of selection conditions.

Mapping of Amp^R and Kan^R recombinants of pML23 showed that all had deleted the $λP_L$ region. Selection for ampicillin resistance allowed all recombinants with acceptor junction endpoints in Sγ3 and in the *Kan* gene to be scored (Fig. 3*A*). Among ampicillin-resistant recombinants recovered from transfection of BJAB, 50% of acceptor junctions mapped within Sγ3. In contrast, among ampicillin-resistant recombinants recovered from transfection of BJAB-B1, 92% of acceptor junctions mapped within Sγ3. Similarly high levels of targeting of recombination within Sγ3 were observed in recombinants recovered from transfection of both EBV-transformed BLs, Akata and Raji. Selection for kanamycin resistance allowed all recombinants with junction endpoints in Sμ and in the *Amp* gene to be scored (Fig. 3*B*). Among

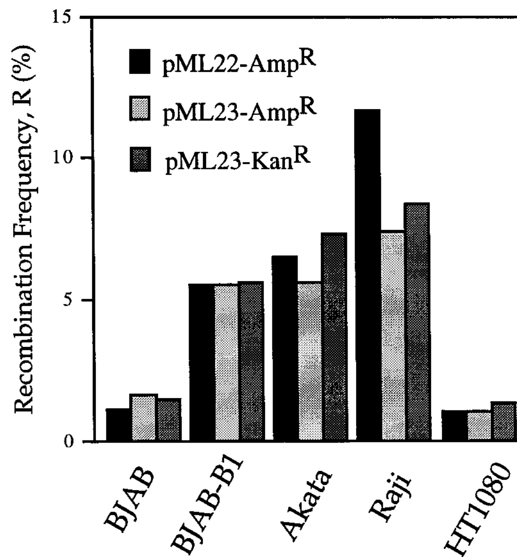

FIGURE 2. Recombination of switch substrates pML22 and pML23. Recombination frequencies (*R*) were assayed in the EBV-negative lymphoma, BJAB; its EBV-infected derivative, BJAB-B1; the EBV-positive BL cell lines Akata (group I) and Raji (group III); and the human fibroblast cell line, HT1080. For each cell line, the left bar represents the substrate pML22, selected for ampicillin resistance; the middle bar represents the substrate pML23, selected for ampicillin resistance; and the right bar represents the substrate pML23, selected for kanamycin resistance. Data are average *R* values determined in three independent transfection experiments.

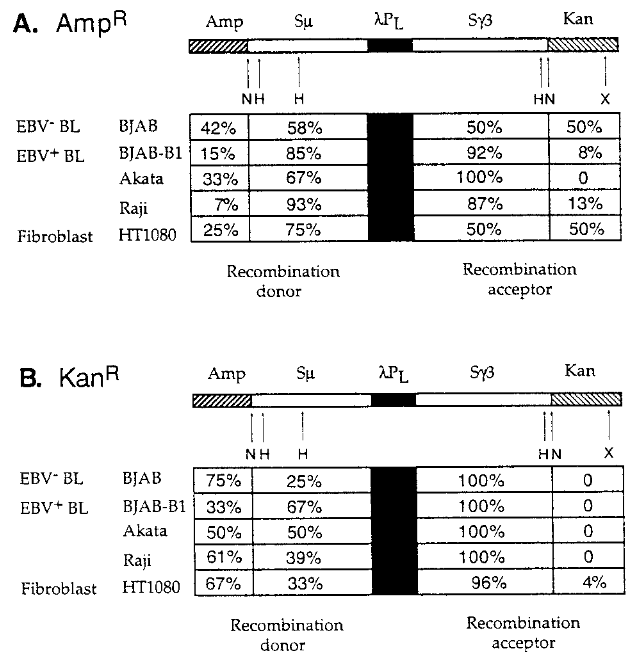

FIGURE 3. Recombination endpoints of pML23 recombinants. Recombinants were selected for ampicillin resistance (*A*) or kanamycin resistance (*B*). In each panel, the top line shows a map of pML23 with cutting sites for *Hin*dIII (H), *Not*I (N), and *Xho*I (X) marked by arrows. Lines below show for each interval the percentage of donor endpoints that mapped within the *Amp* gene or Sμ and the percentage of acceptor endpoints that mapped within Sγ3 or the *Kan* gene. At least 25 recombinants from each line were analyzed.

kanamycin-resistant recombinants recovered from transfection of BJAB, 25% of donor recombination junctions were in Sμ; whereas in recombinants recovered from BJAB-B1, 67% of donor junctions were within Sμ. Targeting of both donor and acceptor endpoints in BJAB-B1 was similar to that observed for other EBV-positive lines, whereas targeting of recombination in the fibroblast, HT1080, resembled that observed in BJAB.

Dunnick et al. (27) have reported that chromosomal switch recombination junctions are heterogeneous, with more than one-third of donor (or upstream) endpoints outside of the Sμ region and ~90% of acceptor (or downstream) endpoints in the appropriate S regions. Recombination within sequences flanking Sμ has also been reported in the CH12F3 lymphoma, which switches from IgM to IgA production after cytokine stimulation (28). Our mapping data show that recombination of pML23 in BJAB-B1 and the EBV-positive BL lines, Akata and Raji, produces junctions similar to those observed during chromosomal switch recombination. Thus, the activities induced by EBV appear to be those that carry out switch recombination in vivo.

Transcriptional activator enhances substrate recombination

Recombination of the extrachromosomal switch substrates in primary murine B cells was shown to be dependent on the presence of transcriptional regulatory regions (5). To ask whether a transcriptional activator can enhance substrate recombination, we assayed recombination of the substrate pML122, which carries the Ig heavy chain intron enhancer and promoter sequences just upstream of Sμ. Table II compares recombination of pML122 and pML22 in transfections conducted in parallel. The recombination frequency of pML122 was 2- to 3-fold higher than that of pML22 in all lines examined (Table II). Moreover, recombination of pML122 in Raji reached a level of $R = 20\%$, comparable with the very high level of substrate recombination evident in transfection of primary B cells activated by LPS with the analogous murine substrate, pHL122 (5).

Mapping showed that acceptor junctions were predominantly in the Sγ3 region in recombinants recovered from transfections of BJAB, BJAB-B1, and Raji (Fig. 4). The transcriptional regulatory region thus enhanced targeting of recombination, as also occurs in transfection of murine cells (6). Donor junctions mapped both within $E\mu P_H$ and within Sμ. A considerable fraction of chromosomal switch junctions produced in vivo also lie upstream of the G-rich Sμ region (27, 28).

EBV genome contributes to LR1 DNA binding activity

LR1 is a B cell-specific, sequence-specific DNA binding protein complex which is induced in primary murine B cells activated for switch recombination (16–19). LR1 has multiple potential duplex DNA binding sites in each of the G-rich switch regions. LR1 is a heterodimer of two subunits, nucleolin and a specific isoform of heterogeneous ribonucleoprotein D, and both subunits bind very tightly to G-G paired structures which may form during transcription of the G-rich S regions. This has led to a working model for switch recombination in which LR1 may participate in synapsis of the two S regions activated for recombination (reviewed in Ref. 29). Because of the clear differences in the levels and targeting of recombination in BJAB and its EBV-positive derivative, BJAB-B1, it was of interest to determine whether LR1 DNA binding activity might correlate with recombination. We therefore isolated nuclear extract from Raji, Akata, BJAB-B1, BJAB, BJE2, and the fibroblast, HT1080, and assayed LR1 DNA binding activity by gel mobility shift. These assays compared binding to two different ^{32}P-labeled duplex synthetic oligonucleotides. One (Sγ1) carried a wild-type LR1 binding site from the murine Sγ1 switch region, and the other (G14A) carried a single base mutation of that site

Table II. *Effect of $E\mu P_H$ transcriptional activator on switch substrate recombination*[a]

Cell Lines	Plasmids	$E\mu P_H$	DH10B Amp^R	DH10B Cm^R	DH10B(λ) Amp^R	DH10B(λ) Cm^R	R (%)	R (avg.)
BJAB	pML122	(+)	179	700	9232	1554	4.3	
			73	613	1616	451	3.3	3.8 ± 0.4
	pML22	(−)	11	870	389	405	1.3	
			58	1372	2868	1014	1.5	1.4 ± 0.1
BJAB-B1	pML122	(+)	192	1095	2024	1084	9.4	
			83	623	608	555	12.2	
			102	650	1158	812	11.0	10.7 ± 0.8
	pML22	(−)	279	1465	3220	878	5.2	
			121	1274	949	636	6.3	
			31	2068	483	996	3.1	4.9 ± 0.9
Akata	pML122	(+)	606	604	2844	558	19.7	
			437	550	3028	482	12.6	
			352	324	2080	315	16.5	16.3 ± 1.7
	pML22	(−)	419	735	8432	677	4.5	
			377	444	4968	303	5.2	
			250	521	2528	408	7.7	5.8 ± 1.0
Raji	pML122	(+)	208	429	1087	452	20.2	
			151	385	628	283	17.6	
			192	520	725	437	22.3	20.0 ± 14
	pML22	(−)	103	372	725	308	11.8	
			76	361	611	195	6.7	
			84	399	821	242	6.2	8.2 ± 1.8
HT1080	pML122	(+)	35	1676	1348	1372	2.1	
			45	890	2943	1120	1.9	2.0 ± 0.1
	pML22	(−)	20	1448	1568	1255	1.1	
			23	450	2835	559	1.0	1.1 ± 0.1

[a] Plasmids pML122 and pML22 were transiently transfected into the cell lines shown. After 42 h, low m.w. DNA was recovered, digested with *Dpn*I, and assayed for recombination. The table shows the number of Amp^R and Cm^R colonies recovered after transformation of DH10B and the isogenic lysogen, DH10B(λ). Each line represents data from a single transfection experiment. The recombination frequency (R) was calculated as the ratio of Amp^R transformants in DH10B to those in DH10B(λ), normalized by the control Cm^R transformants. The rightmost column shows the average recombination frequency (R (avg.)) ± SE.

which decreases binding of LR1 about 15-fold (18). The nonspecific end-binding factor Ku, which is present in all these extracts and binds to both duplexes, provided an internal control on activity of the extracts. As shown in Fig. 5, LR1 DNA binding activity (lower band) was evident in all the EBV-positive cell lines assayed, including Raji, Akata, and BJAB-B1. LR1 DNA binding activity was much reduced in BJAB and its EBNA-2-transfected derivative, BJE2, and was completely absent from the human fibroblast line HT1080. The absence of binding to the G14A duplex (right) confirmed that the observed binding is due to LR1. These data show that enhanced LR1 DNA binding activity is correlated with the presence of EBV genome in the cells and that both levels and targeting of switch substrate recombination correlate with the levels of LR1 DNA binding activity.

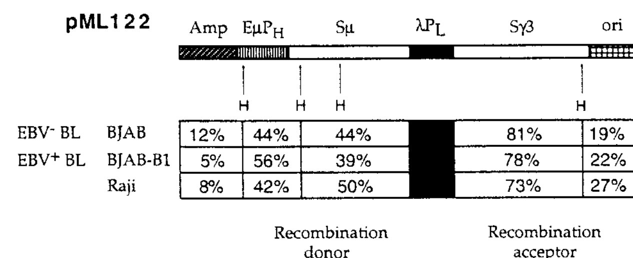

FIGURE 4. Recombination endpoints of pML122 recombinants. Recombinants were selected for ampicillin resistance and mapped by *Hin*dII digestion. Symbols as in Fig. 3.

Discussion

We have found that EBV induces activities that support very active switch recombination in human cell lines. These experiments have used extrachromosomal recombination substrates carried by an SV40-based shuttle vector that supports DNA replication in human cells. Mapping of recombination products showed that these substrates underwent recombination mediated by S region sequences during transfection of EBV-positive human B cell lines. The extrachromosomal switch substrates that we have described here should also be useful in other contexts, because they provide a means for assaying switch recombination activities in a variety of human cell lines.

Comparison of recombination in BJAB and its EBV-infected derivative, BJAB-B1, showed that EBV infection induces recombination activities. The EBV-infected lymphoma, BJAB-B1, supported 5-fold higher levels of recombination of the substrate pML22 than did its EBV-negative parent line, BJAB. Modification of the recombination cassette to include a strong transcriptional activator upstream of Sμ enhanced recombination in both cell lines but did not boost recombination in BJAB to the level observed in BJAB-B1. Recombination was highest in the two BL lines, particularly in Raji. In this group III EBV-transformed BL, recombination levels approached 20% of recovered plasmids, comparable with that observed in transfection of primary murine B cells activated by LPS.

Mapping of recombinants showed that the activities that promote switch substrate recombination in EBV-infected cells are

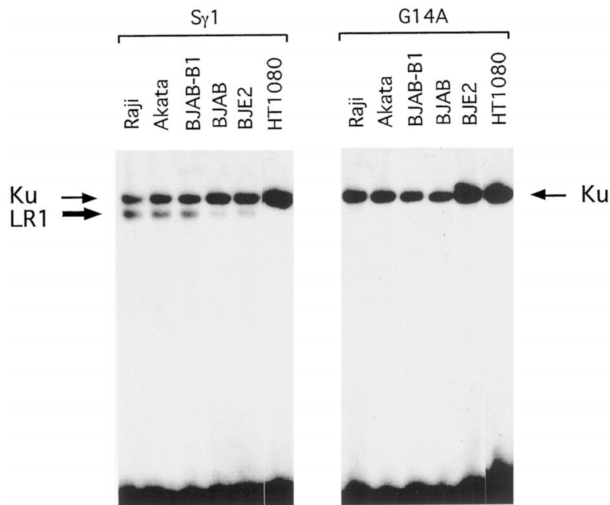

FIGURE 5. Cell type specificity of LR1 DNA binding activity. Gel mobility shift assay of binding by activities in nuclear extracts from the indicated cell lines to ^{32}P-labeled synthetic duplex oligonucleotides containing a wild type Sγ1-binding site (*left*) or mutant G14A binding site (*right*). Protein-DNA complexes were resolved on a 7% polyacrylamide gel. Large arrow, LR1/DNA complex (lower band); small arrows, Ku/DNA complex (upper band).

very similar or identical to those that promote S region recombination in activated B cells. The acceptor endpoints in recombinants were almost exclusively within the Sγ3 region, while a significant proportion of donor endpoints mapped upstream of the Sμ region sequences, as also occurs in chromosomal switch recombination (27, 28). A considerably higher fraction of recombination junctions were targeted within the S regions in recombinants recovered from the EBV-positive lines.

The results reported here show that EBV infection can induce host activities essential to switch recombination. We have assayed for one such activity, LR1, a B cell-specific, sequence-specific duplex DNA binding protein with multiple sites in the G-rich S regions and at the c-*myc* locus (19). In the cell lines examined, switch substrate recombination correlated with levels of LR1 duplex DNA binding activity. LR1 binding activity is absent from resting B cells but is induced by EBV infection or activation of switch recombination. Because EBV infection induces LR1 activity, it is an intriguing possibility that the host factor LR1 plays some role in the EBV life cycle. This possibility is consistent with the fact that there are multiple potential binding site for LR1 in the EBV BamW repeats. In addition, the BamW repeats are frequently sites of EBV genomic reorganization during lytic replication, and just as translocations contribute to human disease, EBV genomic rearrangements have the potential to alter viral gene regulation or gene function (3, 4). EBV therefore appears to induce host activities that diminish stability of the viral genome and are also responsible for the c-*myc* translocations characteristic of sporadic BL.

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