MOLECULAR AND CELLULAR BIOLOGY, Aug. 1981, p. 753-762
0270-7306/81/080753-10$02.00/0

Vol. 1, No. 8

Initiation Points for Cellular Deoxyribonucleic Acid Replication in Human Lymphoid Cells Converted by Epstein-Barr Virus

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Received 6 January 1981/Accepted 27 May 1981

Replicon size was estimated in two Epstein-Barr virus (EBV)-negative human lymphoma lines, BJAB and Ramos, and four EBV-positive lines derived from the former ones by infection (conversion) with two viral strains, B95-8 and P3HR-1. Logarithmic cultures were pulse-labeled with [^{3}H]thymidine, and the deoxyribonucleic acid was spread on microscopic slides and autoradiographed by the method of Huberman and Riggs. After developing, replication forks were visualized as silver grain tracks on the autoradiograms. Average replicon size was estimated by scoring the number of replication forks per constant length of deoxyribonucleic acid and by measuring distances between centers of adjacent tracks, followed by detailed statistical analyses. Three of the four EBV-converted cell lines, BJAB/B95-8, Ra/B95-8, and Ra/HRIK, were found to have significantly shorter replicons (41, 21, 54% shorter, respectively), i.e., more initiation points, than their EBV-negative parents. BJAB/HRIK had replicons which were only slightly shorter (11%) than those of BJAB. However, analysis of track length demonstrated that extensive track fusion occurred during the labeling of BJAB/HRIK, implying that its true average replicon size is shorter than the observed value. The results indicate that in analogy to simian virus 40, EBV activates new initiation points for cellular DNA replication in EBV-transformed cells.

Simian virus 40 (SV40)-transformed cells have more initiation points for deoxyribonucleic acid (DNA) replication (shorter replicons) than do nontransformed cells when grown under normal growing conditions and fewer (longer replicons) when incubated in depleted medium (15, 22). It was suggested that SV40 induces transformation by activating new initiation points for host DNA replication that may escape normal cellular control (14-16). Our experiments with *tsA* mutants indicated that a functional big T-antigen is required. We proposed that big T-antigen of SV40 acts as an initiator of host DNA synthesis and that this action is an important part of the mechanism of transformation by SV40 (16).

Cells that carry the Epstein-Barr virus (EBV) genome also contain a nuclear antigen (EBNA). Although there is no direct evidence, EBNA is considered to be the transforming protein of EBV (11). Like big T-antigen of SV40, EBNA binds with high affinity to cellular DNA (12, 13) and chromosomes (20). It was therefore of interest to study whether EBV also activates new initiation points for cellular DNA replication during transformation.

EBV has been shown to be associated with two human malignancies, Burkitt's lymphoma and nasopharyngeal carcinoma (9, 27). The presence of the EBV genome(s) and EBNA was demonstrated in the great majority of Burkitt's lymphoma cases (97%) and in the permanent lymphoma lines established from them. However, about 3% of the Burkitt lymphoma cases examined were found to be EBV (and EBNA) negative. The permanent cell lines established from these cases were also EBV (and EBNA) negative. These EBV-negative lines can be converted in vitro by infection to EBV-positive lines. The EBV-positive lines are different from their "parent" lines: they exhibit lower serum requirements and grow to higher cell densities (24, 25).

Under laboratory conditions, EBV can be used to infect nondividing human B lymphocytes and transform them into continuous cell lines ("immortalization"; 18). Invariably, these cell lines are EBNA positive. Common sources for lymphocytes in these experiments are peripheral

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blood samples of EBV-negative adult donors or cord blood samples.

For the investigation of initiation points, we have chosen the system of cellular conversion by EBV, which might be analogous to transformation of a "normal" cell line by SV40. This system provides us with cell lines that are presumably isogenic and differ only in the EBV genome (24). The experiments were conducted by analyzing replicon size by using the elegant method of fiber autoradiography of Huberman and Riggs (8). Our results indicate that similar to SV40, EBV activates new initiation points for cellular DNA replication in EBV-converted cells.

MATERIALS AND METHODS

Cells and media. The nonconverted EBV-negative lymphoma lines BJAB (17) and Ramos (10) and their converted EBV-positive derivatives (4, 10) were kindly supplied by George Klein. The cell lines were free of mycoplasma as determined by growth on agar. Cultures were grown in RPMI 1640 medium supplemented with 20% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.).

Labeling procedure and autoradiography. Since lymphoid cells in suspension cultures are fragile, it was essential to avoid centrifugation of the cells before labeling. In our hands, even a gentle centrifugation reduced thymidine uptake during the 8 min pulse. The percentage of cells that became labeled after centrifugation was not reproducible and as low as <5%, compared with 30 to 50% without centrifugation.

Logarithmic cultures, with a viable count of >95% (determined by the trypan blue exclusion test) were used. Cells were grown in 2 ml of medium in test tubes, at 0.75×10^6 to 1×10^6 cells per ml. The medium was gently sucked off the cell pellet and replaced by 0.5 ml of medium (25% fetal calf serum) containing 5-fluorodeoxyuridine at 2×10^{-6} M. The cells were gently mixed and incubated at 37°C for 30 min. Then [^{3}H]thymidine (~50 Ci/mmol) was added to a final concentration of 500 μCi/ml and the cells were mixed again and incubated at 37°C for 8 min. Labeling was terminated by adding 5 ml of RPMI 1640 (25% fetal calf serum) at 4°C and centrifuging (1,000 rpm, 5 min). The cells were washed twice with cold phosphate-buffered saline and treated with hypotonic solution (75 mM KCl) for 10 min at room temperature to facilitate spreading of the DNA. A drop of cell suspension (containing 1×10^4 to 2×10^4 cells) was then placed on microscopic slides precoated with 1% bovine serum albumin. The cells were lysed by adding a drop of lysis buffer (15), and the DNA was spread on the slides, fixed, washed, and autoradiographed essentially as described before (15).

Analysis of the autoradiograms. Analysis of the autoradiograms was performed as described previously, after 6 months of exposure (15). The slides were examined under ×400 enlargement in a Zeiss microscope equipped with an eyepiece with a ruled engraving. Magnification was determined by using a slide with a 5-mm scale ruled to 1-μm divisions (Zeiss). The slides were coded, and scanning and measurements were performed "double blind," to eliminate possible bias of the investigators. Scanning was done as described before (15), and all of the suitable sets of track that were encountered were scored (in the first preliminary experiments) or photographed (in the detailed experiments described below). Most of the rules have been described (15). The sets of tracks that were scored or photographed had to be straight, had to be well separated, had to correspond to single-fiber width, and had to contain at least three tracks. The tracks had to be longer than their width. In addition, the photographed sets of tracks had to be at least 50 mm (67 μm) long. The reason for this rule was that many of the tracks and intertrack distances were very short (Fig. 1), and visually, it was easier to pick stretches with many short tracks and to overlook long distances between tracks. After all of the slides were scanned, the photographs of the six cell lines were shuffled and measured blind (15), and distances between track centers, and also track lengths, were measured. Because replication is bidirectional, an even number of intertrack distances was recorded for each set of tracks (see legend to Fig. 1, reference 15). If the number of intertrack distances in a set was odd, the two outer distances on each side were averaged and recorded as a single measurement. All measurements were recorded to the nearest millimeter on the photograph, corresponding to 1.3 μm at the fiber level.

RESULTS

Replicon size was analyzed in two EBV-negative lines, BJAB and Ramos, and in each of these lines converted by two strains of EBV, derived from the producer cell lines B95-8 and P3HR-1. Logarithmic cultures of randomly growing cells were labeled for 8 min with high-specific activity [^{3}H]thymidine. The cells were gently lysed, and the DNA was spread on microscopic slides and autoradiographed by a modification of the procedure of Huberman and Riggs (8). By this method, one can observe replication forks along the DNA fiber that became labeled during the radioactive pulse (Fig. 1). Autoradiography of nonlysed cells showed that 30 to 50% of the cells became labeled in the different experiments. There was no apparent difference between the converted and the nonconverted lines.

Measurements of intertrack distances. Our first set of experiments was performed by scoring the number of replication forks per 105 μm of DNA fiber (data not shown). Those experiments indicated that BJAB/B95-8 (converted by the EBV strain B95-8) had distinctly shorter replicons than did BJAB, its parental nonconverted line, and similarly, that Ra/HRIK (converted by the EBV strain P3HR-1) had shorter replicons than did its parental nonconverted line, Ramos. Ra/B95-8 (Ramos converted

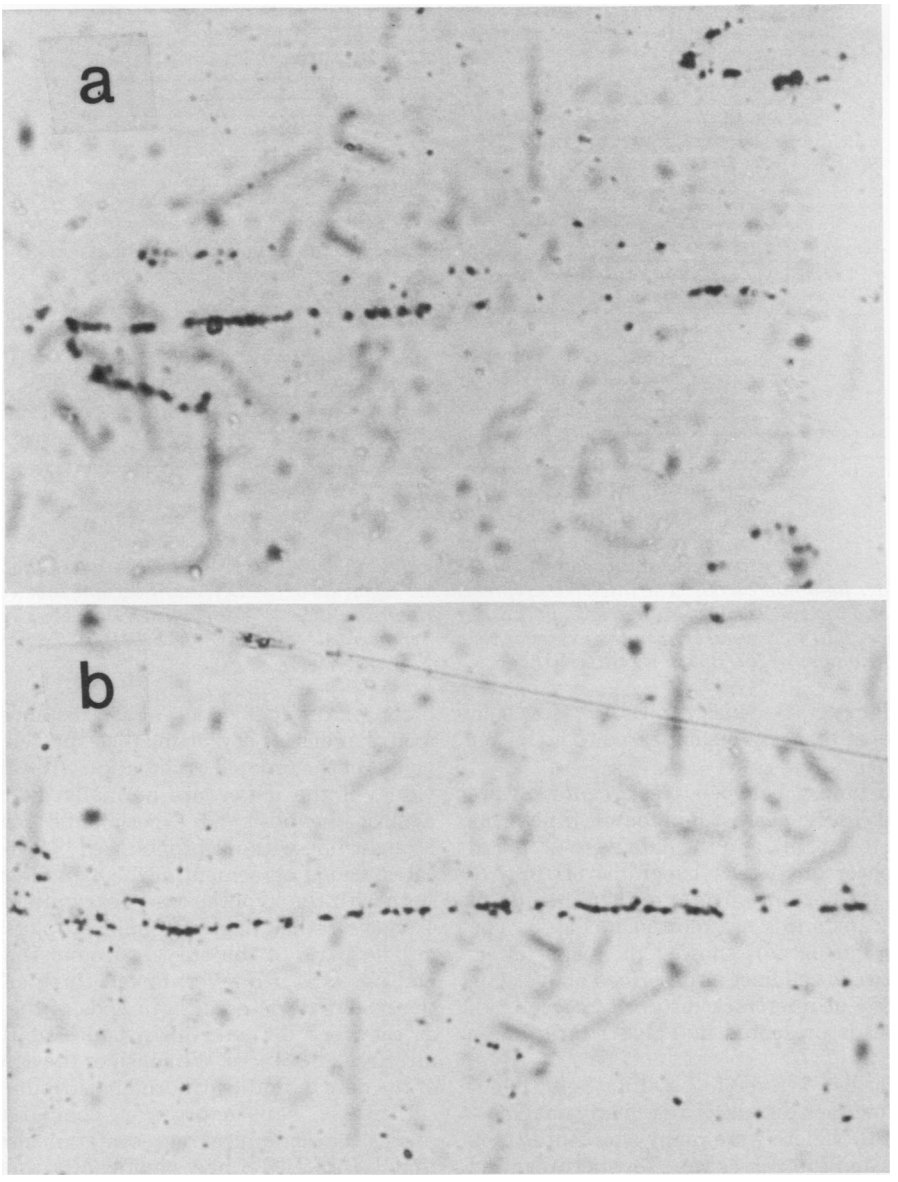

FIG. 1. *Fiber autoradiograms of lymphoid cells. The sets of tracks correspond to segments of the DNA that became labeled during an 8-min pulse with [^{3}H]thymidine. The DNA was spread on microscopic slides and exposed to photographic emulsion for 6 months. The slides were viewed under a magnification of ×400. (a) BJAB; (b) BJAB/B95-8.*

by B95-8 EBV) showed a replicon size only slightly shorter than that of Ramos. BJAB/HRIK (converted by the strain P3HR-1) was not analyzed in those experiments.

We proceeded to study replicon size more precisely in the converted and nonconverted lines. Cultures were labeled, and fiber autoradiograms were prepared, scanned, and photographed as described above. Typical fibers are represented diagrammatically in Fig. 2. Although for each cell line there were fibers with different fork densities, there were apparent differences between the populations of fibers of BJAB and BJAB/B95-8, and between those of Ramos and Ra/HRIK, in agreement with the preliminary experiments. Also in agreement with those experiments, the fork density of Ra/B95-8 was closer to that of Ramos. BJAB/HRIK

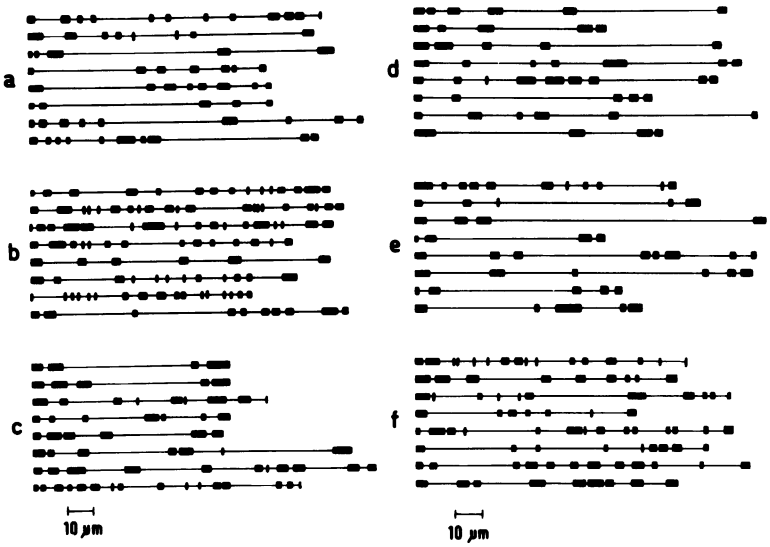

FIG. 2. *Diagrammatic representation of sets of tracks. The thin lines represent DNA fibers that are not visible under light microscopy. The thick bars represent the segments of the DNA that became labeled during the pulse and appear as silver grain tracks in the autoradiograms (Fig. 1). For each cell line, eight randomly picked fibers that were measured in the experiments are represented. (a) BJAB; (b) BJAB/B95-8; (c) BJAB/HRIK; (d) Ramos; (e) Ra/B95-8; (f) Ra/HRIK.*

was similar in track density to BJAB. However, the tracks of this cell line appeared to be longer (see below).

The distances between track centers were measured (to the nearest millimeter, which corresponds to 1.3 μm), and the data were prepared for computer analysis. Histograms of the frequencies (as percent of total measurements) of intertrack distances are plotted in Fig. 3. The frequency histograms showed the same differences between cell lines as described above. The mean value of intertrack distance for each cell population is presented in Table 1 (arithmetic mean).

As described before (1, 7, 8, 15), the distribution of intertrack distances was clearly not Gaussian (Fig. 3). There were many short intertrack distances and the curves were distinctly skewed to the right, to the values of longer distances. Also, the median was much smaller than the (arithmetic) mean, which is another indication of deviation from normality. In our analysis of replicon size of hamster and mouse cells (15, 22), we found that the distributions of intertrack distances could be approximated by lognormal distributions, and therefore, it was more meaningful to compare the geometric means (antilogs of the means of the logs of intertrack distances). Analysis of the present data showed that they could also be approximated by a normal distribution after logarithmic transformation.

Figures 4 and 5 present normal probability plots for the logs of intertrack distances. This analysis consists of plotting the expected normal values of the ordered observations (the expected values if the data were normally distributed) against the observed experimental results. A straight line without pronounced "jumps" indicates a good agreement of the experimental results with the hypothesized distribution. Figure 4 shows rather linear probability plots in the middle parts of the curves, around the means and medians. However, for all three cell lines, the results deviated at both ends of the curves, at the very low and high values of (logs of) intertrack distances. We consider the agreement of the data with the lognormal distribution to be sufficiently satisfactory for comparisons of means. This graphical representation also shows that BJAB/B95-8 had significantly shorter replicons than BJAB. Interestingly, the results for BJAB/HRIK were close to those of BJAB at the lower part and approached those of BJAB/B95-8 at the upper part of the curve. This finding will be discussed below.

A similar analysis for Ramos and derivatives (Fig. 5) also showed approximately straight lines in the middle parts and deviations at both ends of the curves. The curve for Ra/HRIK was shifted significantly to the left (shorter intertrack distances) compared with that for Ramos. The curve for Ra/B95-8 ran closer to that of Ramos and also to its left.

Since agreement with the hypothesized distri-

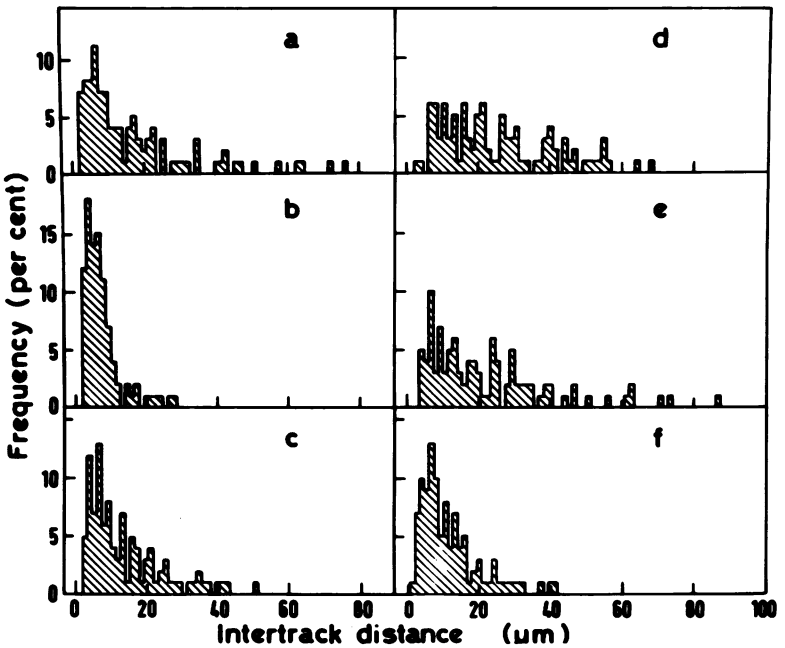

FIG. 3. *Frequency histograms of intertrack distances. Autoradiograms of the six cell lines, labeled for 8 min, were developed after 6 months of exposure. Photographs were taken, and intertrack distances were measured. The frequency, as the percentage of total frequency, of intertrack distances of length x_i is plotted versus x_i. The number of intertrack distances measured for each cell line appears in Table 1. (a) BJAB; (b) BJAB/95-8; (c) BJAB/HRIK; (d) Ramos; (e) Ra/B95-8; (f) Ra/HRIK.*

TABLE 1. *Intertrack distances in EBV-negative and EBV-positive cells*

Cell line	No. of intertrack distances measured	Intertrack distances (μm)		
		Arithmetic mean	Median	Geometric mean[a]
BJAB	118	16.3	10.1	11.5
BJAB/B95-8	204	8.7	5.8	6.8
BJAB/HRIK	156	13.3	9.0	10.2
RAMOS	102	25.2	20.7	20.5
Ra/B95-8	128	21.3	16.3	16.2
Ra/HRIK	302	12.5	8.3	9.5

[a] Geometric means are antilogs of the means of the logs of the intertrack distances.

bution was not perfect, we have tried other analyses of the data as well. (Information available from the authors upon request). In all cases, the analyses led to similar qualitative differences between the cell lines. We have summarized the arithmetic means, medians, and geometric means in Table 1. We consider the geometric means more meaningful than the arithmetic means, because their values are closer to those of the medians and because the middle parts of the normal probability plots for the log distributions are rather straight (Fig. 4 and 5), indicating that the geometric means are reasonably good representatives of the data. The intertrack distances were compared for significance of difference by using t tests on the means of logarithms. Probabilities that the samples represent populations with intertrack distances of the same size are listed in Table 2. Probability values below 0.05 are customarily taken to indicate a significant difference. The analysis showed that the mean intertrack distance for BJAB/B95-8 was significantly different from that of BJAB. The mean intertrack distances of Ra/B95-8 and of Ra/HRIK were significantly different from that of Ramos. By another statistical method (the coefficient of variation approach), the difference between BJAB/HRIK and BJAB was significant at the 10% level (probability value of 0.09).

Measurements of track length. In our previous studies, we found no differences in the average track length between nontransformed and SV40-transformed cells (15). However, it appeared from the photographs that the average track length for BJAB/HRIK was greater than that of BJAB and most of the other cell lines. We proceeded to measure and analyze these lengths.

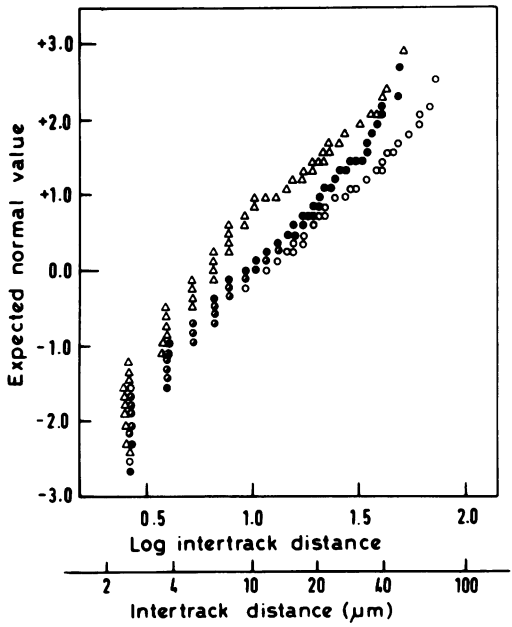

FIG. 4. *Normal probability plots for the logs of intertrack distances of BJAB and its EBV-carrying derivatives. This analysis consists of plotting the expected normal values (the expected values if the data were normally distributed) against the actual experimental results, i.e., logs of intertrack distances. Symbols:* ○, *BJAB;* △, *BJAB/B95-8;* ●, *BJAB/HRIK.*

Figure 6 shows the frequency distribution (percent of total frequency) of track lengths for the six cell lines. BJAB/B95-8 appeared similar to BJAB, with a minor shift to shorter forks. However, the curve for BJAB/HRIK was significantly skewed to higher values of track length. The asymmetry of this curve suggests an extensive merging of tracks. This could have happened whenever adjacent replicons were fully replicated during the pulse so that their converging tracks merged together. A similar situation was described by Callan (3) and by Blumenthal et al. (1). Those authors observed a shift from an almost normal distribution of track lengths (similar to Fig. 6a and b) to an asymmetric distribution of pronounced skewness (Fig. 6c) with increasing pulse length.

The frequency distributions for Ramos and derivatives are somewhat skewed to the right (Fig. 6d to f). However, the three curves are similar to one another.

Table 3 summarizes the results for the track lengths. It is noteworthy that the modal values were identical for all cell lines. We take this to indicate that the rate of fork movement remains unchanged by the EBV conversion. Presumably all the longer tracks that we measured arose from fusion. The rate of chain elongation calculated from the modes was 0.33 μm/min, and from the geometric means, 0.37 to 0.50 μm/min.

DISCUSSION

We have studied replicon size in the EBV-negative lymphoid lines BJAB and Ramos and in their EBV-carrying derivatives after conversion in vitro. We have chosen these cell lines for our studies because the only known genomic difference between the nonconverted and the

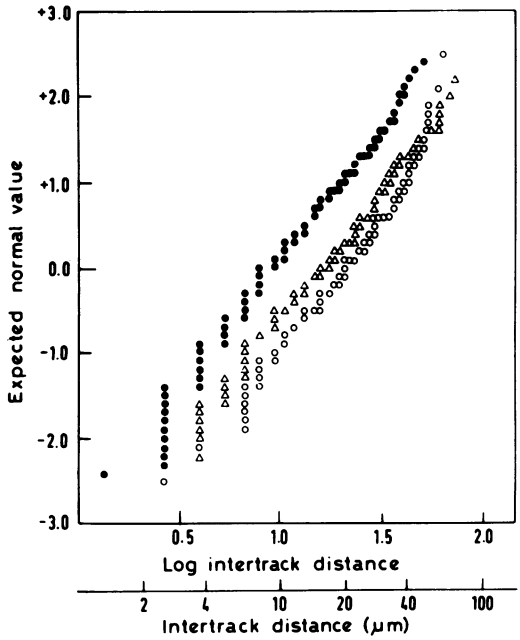

FIG. 5. *Normal probability plots for the logs of intertrack distances for Ramos and its EBV-carrying derivatives. See legend to Fig. 6. Symbols:* ○, *Ramos;* △, *Ra/B95-8;* ●, *Ra/HRIK.*

TABLE 2. *P values for pairwise t tests between the means of the logarithmic distributions*[a]

Cell line	P value			
	BJAB	BJAB/B95-8	Ramos	Ra/B95-8
BJAB/B95-8	<0.0005			
BJAB/HRIK	0.231	<0.0005		
Ra/B95-8			0.016	
Ra/HRIK			<0.0005	<0.0005

[a] Student's *t* tests were performed on the means of the logs of the intertrack distances. Pooled variances were used, since the variances of the populations appeared to be equal. The *P* values correspond to two-sided tests of significance, i.e., tests of inequality in either direction. A *P* value <0.050 indicates a significant difference.

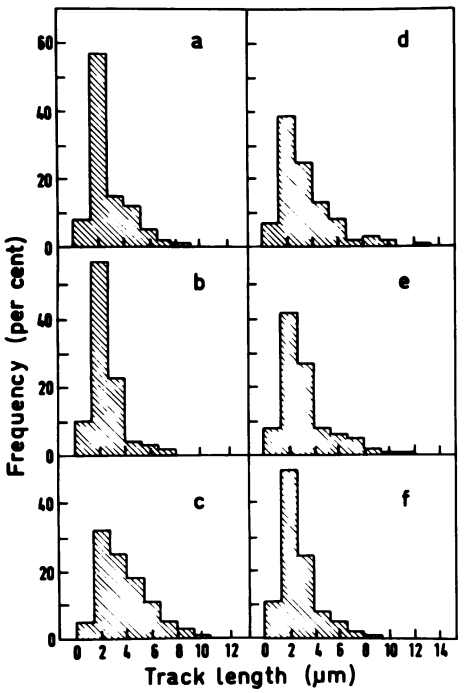

FIG. 6. *Frequency histograms of track lengths. Autoradiograms were prepared as described in the legend to Fig. 3 and photographed, and track lengths were measured. The frequency, as the percentage of total frequency, of tracks of length x_i is plotted versus x_i. The number of track lengths measured for each cell line appears in Table 3. (a) BJAB; (b) BJAB/B95-8; (c) BJAB/HRIK; (d) Ramos; (e) Ra/B95-8; (f) Ra/HRIK.*

TABLE 3. *Track length in EBV-positive and EBV-negative cells*

Cell line	No. of track lengths measured	Track length (μm)			
		Arithmetic mean	Mode	Median	Geometric mean[a]
BJAB	252	3.5	2.7	3.0	3.2
BJAB/B95-8	260	3.2	2.7	3.0	3.0
BJAB/HRIK	324	4.4	2.7	4.1	4.0
RAMOS	315	4.1	2.7	3.6	3.7
Ra/B95-8	248	3.9	2.7	3.3	3.5
Ra/HRIK	323	3.4	2.7	3.1	3.1

[a] Geometric means are antilogs of the means of the logs of the track lengths.

converted lines is the presence of the EBV-genome. However, the converted cells acquired higher resistance to cell density and reduced serum dependence (25). The converted cell lines have the same karyotype as the nonconverted ones (26; J. Zeuthen, personal communication). Therefore, a decrease in replicon size cannot be attributed to a selective loss of DNA with longer replicons.

We have estimated replicon size in EBV-converted and nonconverted cells by the method of fiber autoradiography. Our previous studies (15; unpublished data) showed that this method is the most suitable for measuring replicon size. The limitations of this procedure have been discussed in detail before (1, 7, 8, 15). An additional limitation of the present study was the abundance of many short intertrack distances in these lymphoid lines, which were sometimes masked by track fusion (see below). To circumvent these difficulties, a strict set of rules was observed (see above) and the experiments were conducted double blind. We believe that systematic errors inherent in the technique were the same for all of the cell lines (with the exception of BJAB/HRIK, where extensive track fusion has occurred, as discussed below) and that comparisons are therefore valid.

The results and the three statistical measures computed showed an "average" intertrack distance which is much shorter for the EBV-positive lines BJAB/B95-8 and Ra/HRIK and moderately shorter for Ra/B95-8 than for their respective EBV-negative parental lines (Table 1). The differences between those cell lines were highly significant (Table 2). Shorter intertrack distances represent shorter replicons and more initiation points. Since the only known genomic difference between the converted and nonconverted lines is the presence of EBV DNA (24), our findings indicate that EBV is responsible for activation of new initiation points for cellular DNA replication in BJAB/95-8 and in Ra/HRIK.

The only EBV-positive line that did not exhibit significantly more initiation points is BJAB/HRIK. However, track length analysis (Fig. 6) suggested that extensive track fusion has taken place in this cell line during the labeling. We would like to suggest that the true value for an average replicon for BJAB/HRIK is distinctly shorter than the observed, and significantly shorter than that of BJAB.

Support for this suggestion comes from the statistical analyses. The normal probability plot for BJAB/HRIK resembled that of BJAB at the low values and approached that of BJAB/B95-8 at the high values of intertrack distances (Fig. 4), suggesting that the main difference between the two EBV-converted BJAB lines is the abundance of very short replicons in BJAB/B95-8, which have been presumably lost by track fusion in BJAB/HRIK. Note also the higher sample size (204 intertrack distances) of BJAB/B95-8 compared with BJAB/HRIK (Table 1). We measured 91 intertrack distances shorter than 5.3 μm in BJAB/B95-8 and only 37 in BJAB/HRIK (27 in BJAB). The number of distances

longer than 5.3 μm was almost the same for BJAB/B95-8 (113) and BJAB/HRIK (119).

It should be also pointed out that the logarithmic transformation that we have used in the analysis for significance of differences (Table 2) gives more weight to the shorter intertrack distances, and therefore a bias due to track merging and preferential loss of short distances would be magnified. By the coefficient of variation approach, which gives equal weight to the longer and shorter intertrack distances, the difference between BJAB/HRIK and BJAB was significant at the 10% level. Also, compare the difference between the arithmetic means to that of the geometric means for these cell lines (Table 1). For all of these reasons, we suggest that in BJAB/HRIK also, EBV-activated initiation points are functioning.

It is hard to estimate from these experiments what the true value for replicon size for BJAB/HRIK is. We cannot use a shorter labeling time to resolve this question because a shorter pulse results in autoradiograms with many tracks that are too short and cannot be distinguished with confidence from the background.

From the results of Table 1, we calculated the size for an "average" replicon, which we define as twice the geometric mean, assuming that replication is bidirectional. The sizes ranged from 13.6 μm for BJAB/B95-8 to 40.9 μm for Ramos (Table 4). It is interesting to note that the two EBV-negative lines BJAB and Ramos, both isolated from patients with similar B lymphomas, were so different in their average replicons; 23 μm for BJAB and 41 μm for Ramos. The two cell lines differ also in their growth properties: BJAB grows more vigorously in tissue culture and is less sensitive to high cell density and serum starvation (A. Oppenheim and H. Ben-Bassat, unpublished data). We do not know whether these differences are related to the difference in replicon size.

Assuming that the additional initiation points in the converted cells are EBV activated, it is remarkable that both BJAB/B95-8 and Ra/HRIK had almost the same number of additional initiation points per 1.0 mm of DNA: 30.0 for BJAB/B95-8 (73.6 − 43.6 = 30.0) and 28.5 for Ra/HRIK (52.9 − 24.4 = 28.5). Comparing these two lines, we did not see, therefore, a difference in the activation of new initiation points by the two EBV strains derived from B95-8 and P3HR-1. We also did not see a difference between the two cell lines BJAB and Ramos. If EBV is using specific nucleotide sequences of the cellular DNA for the activation, these sequences may be equally accessible in the two cell lines. By a similar calculation, the number of EBV-activated initiations per 1.0 mm of DNA in Ra/B95-8 was only 6.5 (30.9 − 24.4 = 6.5). However, note that the total number of initiations of DNA replication for Ra/B95-8 was 30.9 per 1.0 mm of DNA, which is very close to the number of initiation points that were presumably activated by EBV in BJAB/B95-8 and in Ra/HRIK (30.0 and 28.5 initiations per 1.0 mm of DNA, respectively). It is therefore conceivable that in Ra/B95-8, only the EBV-activated initiations were functioning. An analogous situation was observed in SV40-transformed Chinese hamster lung (CHL) cells after isoleucine depletion (15). It appeared that only the SV40-activated initiations remained functioning after the amino acid starvation. The nontransformed CHL cells stopped DNA replication almost completely (only 1 to 3% of the cells became labeled during 8 min of [^{3}H]thymidine pulse), whereas the SV40-transformed cells continued to replicate their DNA (about 50% of the cells became labeled), but from fewer initiations. Those results suggested that the SV40-activated initiation of DNA replication can bypass normal cellular control signals and allow transformed cells to enter S phase also under unfavorable growth conditions (15). We would like to suggest that also in Ra/B95-8, the original "normal" initiations were not functioning, possibly because the normal initiator that functions in Ramos had been lost. This explanation is plausible in view of the greater sensitivity of Ramos to unfavorable growth conditions (high cell density and low serum) (Oppenheim and Ben-Bassat, unpublished data).

A striking feature of the autoradiograms of these cell lines is the abundance of very short replicons. Regardless of the average replicon size, we found stretches of DNA with clusters of tracks that are less than 1 μm apart in all of the cell lines. We observed a similar track pattern also in normal cord blood lymphocytes treated

TABLE 4. *Replicons of EBV-converted and nonconverted cells*

Cell line	"Average" replicon (μm)[a]	Initiations per 1.0 mm of DNA
BJAB	22.9	43.6
BJAB/B95-8	13.6	73.6
BJAB/HRIK	20.5	48.8
Ramos	40.9	24.4
Ra/B95-8	32.4	30.9
Ra/HRIK	18.9	52.9

[a] We define an "average" replicon as twice the geometric mean of the intertrack distances, since replication is bidirectional.

with protein A, a mitogen for human B lymphocytes (A. Oppenheim and J. Zeuthen, unpublished data). Therefore, we think that this pattern is not related to the malignant state of the lines used in the present study, but to their tissue origin. Studies of human fibroblasts (A. Oppenheim, unpublished data) show track patterns that are similar to those of hamster and mouse fibroblasts (15, 23). Tissue-specific differences in replicon size have been observed before (2), implying that potential sites for initiation of replication are not always utilized. A similar conclusion is reached from variations in replicon size that result from growth conditions (6). Alternatively, it is possible that sites for initiation on the DNA are not sequence specific, and are a function of the state of local condensation of the chromatin (R. G. Martin, Adv. Cancer Res., in press). Interestingly, in spite of the abundance of short replicons, human lymphocytes appear to have an S phase of 5 h or more (5), similar in duration to that of fibroblasts.

EBNA, which is present in all of the EBV-transformed cells, has been implicated as the transforming protein of this virus (11). This antigen has been shown to bind with high affinity to DNA (12, 13) and to chromosomes (20). Microinjection of purified EBNA induced DNA synthesis in quiescent mouse 3T3 cells (11; J. Zeuthen, personal communication). It is tempting to speculate that EBNA, in analogy to T-antigen of SV40, acts as an initiator of cellular DNA replication in the EBV-transformed cells. It is also noteworthy that DNA-binding antigens, which are presumably nuclear, were detected in cells transformed by other DNA-transforming viruses, herpesvirus papio and herpesvirus ateles (19, 21).

ACKNOWLEDGMENTS

We are grateful to N. Goldblum for encouragement, support, and valuable discussions. We thank G. Klein for supplying the cell lines and for his continuous interest. We are also grateful to G. Nathan, S. Oman, Y. Wax, and I. Einot of the Department of Statistics, the Hebrew University, for consultation, invaluable suggestions, and assistance. We are indebted to M. Wormser for the mycoplasma test and to R. Sadeh for typing the manuscript.

One of us (A.O.) was supported, in part, by the Israel Cancer Research Fund.

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