Induction of Sister Chromatid Exchange by Polyoma Large Viral Tumor Antigen in Transformed Rat Fibroblasts[1]

Eric H. Brown and Claudio Basilico

Department of Pathology, New York University School of Medicine, New York, New York 10016

ABSTRACT

The frequency of sister chromatid exchange (SCE) was determined in rat fibroblasts transformed by wild-type polyoma virus or by a mutant temperature sensitive for viral large tumor antigen function (ts-a). Elevated SCE frequencies were observed in two wild-type transformed cell lines growing at 37° and in four ts-a-transformed lines upon growth at the permissive temperature for large viral tumor antigen (33°). The increase in SCE frequency in ts-a-transformed cells at 33° was reversed by growth at 39° (nonpermissive for T-antigen function). An increase in SCE at 33° was not observed in untransformed cells or in a ts-a-transformed cell line which makes a defective large viral tumor antigen. These results suggest that large viral tumor antigen can induce SCEs. Since large viral tumor antigen is also responsible for amplification of integrated viral DNA sequences (4), we tried to correlate this phenomenon with the increased SCE frequency. However, increasing SCE artificially by growing cells in the presence of 12-O-tetradecanoylphorbol-13-acetate did not result in amplification of integrated viral DNA in the absence of large viral tumor antigen function. Thus, there is no simple causal relationship between increased SCE and amplification.

INTRODUCTION

Transformation of rat cells by PY[2] virus requires the integration of the viral genome into the host DNA. It has recently been shown that, in the presence of a functional large viral tumor (T) antigen, integrated viral sequences can be amplified to generate long tandem repeats (4). The mechanism by which amplification occurs is unclear. One possibility is that amplification results from a recombinational event such as an unequal SCE within the viral DNA sequences. Such a mechanism is believed to be responsible for tandem duplication of the bobbed and bar loci in *Drosophila* (13, 17).

SEC could be a measure of recombinational activity, and the frequency of SCE has been shown to increase when cells are transformed by SV40 (12) and Rauscher leukemia virus (3). Thus, the amplification of integrated viral sequences in PY-transformed cells may result from an increased frequency of recombinational events (as evidenced by increased SCE) following transformation. We therefore attempted to determine the effect of large T-antigen, which is required for amplification, on the frequency of SCE in PY-transformed cells. We approached this problem by observing the frequency of SCE in several cell lines transformed by the ts-a PY mutant (7) which produces a temperature-sensitive large T-antigen.

These ts-a-transformed cell lines offer the advantage of being phenotypically transformed at both the permissive (33°) and nonpermissive (39°) temperatures for large T-antigen function (2). Thus, it is possible to observe the effects of T-antigen on SCE in the absence of other detectable phenotypic variation. We found that, under conditions permissive for large T-antigen function, an increase in SCE was observed in 4 cell lines.

MATERIALS AND METHODS

Cells. The general properties of F2408 Fischer rat cells transformed by PY virus have been described previously (14, 18). PY 53 and 55 are PY wt transformants. Rat cell lines ts-a H3A, ts-a H6A, ts-a 9, and ts-a R51 were transformed by the ts-a mutant of PY virus (7) and have been described previously (4, 6, 8). Ts-a H3* is an evolutionary derivative of ts-a H3A that produces a truncated large T-antigen (5).

Cells were grown in Dulbecco's modified Eagle's medium containing 10% calf serum. Untransformed F2408 and PY 53 and 55 were grown at 37° unless otherwise stated. Cell lines transformed by PY ts-a mutants were maintained at the nonpermissive temperature for T-antigen function (39°).

SCE. Cells were grown in the presence of 5-bromodeoxyuridine for 2 cell generations. The cells were then exposed to Colcemid for 3 hr and trypsinized, and metaphase spreads were prepared (1). In order to obtain differentially stained chromatids, a modification of the Giemsa technique described by Schneider *et al.* (15) was utilized. Briefly, cells were stained with Hoechst 33258 dye (50 μg/ml) for 15 min. The slides were drained and mounted with 0.095 citrated phosphate buffer, pH 8.0, and were exposed to light from one 400-watt General Electric fluorescent tube at a distance of 22 cm for 3 hr followed by staining with Giemsa (Fisher; 2% in phosphate buffer, pH 6.8).

RESULTS

The frequency of SCE in normal rat F2408 cells and wild-type PY-transformed cells was compared to determine whether transformation affected this parameter. In 2 PY-transformed cell lines (PY 53 and 55), an increase in frequency of SCE was observed relative to F2408 cells (Chart 1). For example, in PY 53 and 55, a majority of the cells (67 and 75%, respectively) had an average SCE/chromosome frequency greater than 0.2. In contrast, only 40% of the F2408 cells exhibited an SCE frequency in this range. These results suggested that transformation by polyoma is accompanied by increased SCE, but the difference observed was not very striking. A possible reason for the apparent heterogeneity of the transformed populations with respect to SCE could have been the fact that such cells undergo a high rate of rearrangements (2, 4, 5) and evolve towards a suppression of large T-antigen function (5, 10). Since these phenomena cannot be controlled in wt transformed cells, we performed all subsequent experiments in cells transformed by the ts-a mutant of PY virus that produces a thermolabile large T-antigen. This allowed us to study a more carefully
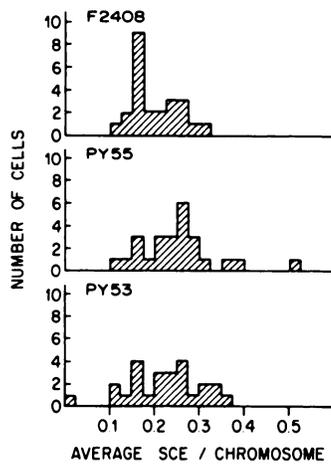
Chart 1. Frequency of SCE in normal and PY-transformed rat fibroblasts. Twenty-five randomly chosen metaphase spreads were scored for the number of SCE. Since there was some variability in the chromosome number in different spreads, the data were expressed as the average number of SCE per chromosome in each cell.

controlled situation since at 39° (where large T-antigen is nonfunctional), the integrated arrangement of PY DNA is stable (2, 4, 5).

We therefore compared the SCE frequency in ts-a-transformed cell lines growing at the permissive (33°) and nonpermissive (39°) temperatures for T-antigen function. As shown in Chart 2 and Table 1, 2 cell lines, H6A and H3A, underwent a gradual increase in the frequency of SCE when grown at 33° (Fig. 1). It was also found that, upon shifting the cells grown at 33° back to 39°, there was a decrease in SCE. Similar results were obtained in 2 other ts-a-transformed cell lines, R5-1 and ts-a 9 (data not shown). These results indicated that an increase in SCE was observed only under conditions in which T-antigen is active. A return to conditions in which T-antigen is nonfunctional results in a decrease in SCE. It has also to be noted that, while H3A and H6A contain a functional T-antigen at 33° and produce free viral DNA molecules (8, 17), this is not the case with the ts-a R5-1 and the ts-a 9 lines. These lines contain less than a single-copy insertion of viral DNA and, although encoding a functional viral large T-antigen, do not spontaneously produce free viral DNA, presumably because intramolecular homology is necessary for this phenomenon to occur at an appreciable rate.[3] The increase in SCE observed, therefore, does not seem related to the presence in these cells of free viral DNA molecules.

We considered the possibility that growth at 33° in itself, rather than T-antigen, might be responsible for the observed increase in SCE. To answer this question, F2408 and ts-a H3*, a transformed cell line which produces a truncated form (~85K), of large T-antigen, nonfunctional even at low temperature (5), were grown at 33°, and the SCE frequency was examined. Neither one of these 2 cell lines displayed an increase in SCE after 1 month at the lower temperature (Chart 3; Table 1). Thus, growth at 33° has no effect on SCE independent of T-antigen function.

Since the increase in SCE seems to be dependent on the same conditions which allow amplification, we attempted to determine whether a correlation existed between amplification

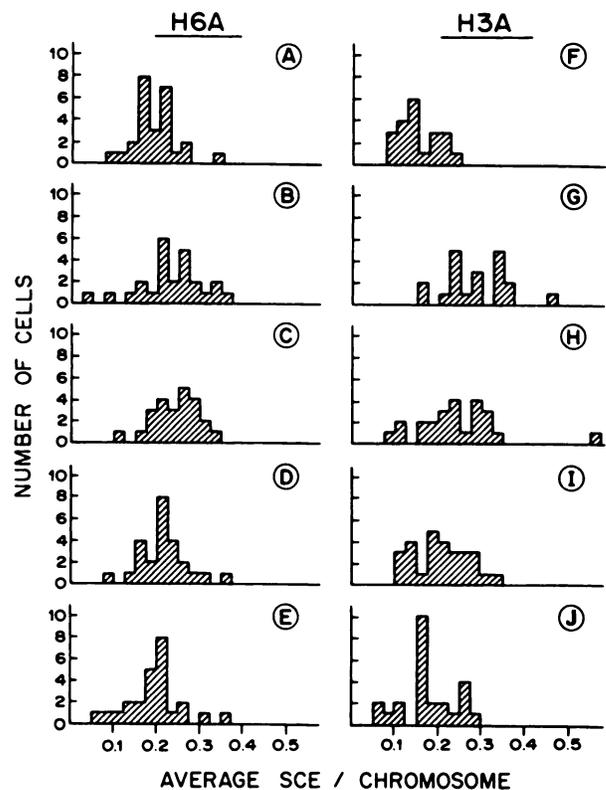[3] V. Colantuoni, Dailey, L., and Basilico, C., manuscript in preparation.

Chart 2. Frequency of SCE in ts-a PY-transformed cells grown at the permissive and nonpermissive temperature for T-antigen function. Twenty to 25 metaphase spreads were scored. The SCE frequency is shown in H6A cells grown at 39° (A), at 33° for 4 weeks (B), 7 weeks (C), and 7 weeks followed by growth at 39° for 1 week (D) and 3 weeks (E). Similarly, SCE frequency is shown in H3A cells grown at 39° (F), 33° for 2 weeks (G), 9 weeks (H), 7 weeks followed by growth at 39° for 3 days (I), and 9 weeks followed by 1 week at 39° (J).

Table 1

Frequency of SCEs in normal and ts-a PY-transformed rat cells

Cell line	Growth temperature	Time after shift to 39°	Mean SCE/ chromosome[a]	p[b]
F2408	37°, 39°		0.19 ± 0.04	
	33 (6)[c]		0.19 ± 0.04	
H6A	39		0.19 ± 0.01	
	33 (4)		0.23 ± 0.01	< 0.02
	33 (7)		0.25 ± 0.01	< 0.001
	33 (7)	1 wk	0.21 ± 0.01	> 0.1
	33 (7)	3 wks	0.20 ± 0.05	> 0.5
H3A	39		0.16 ± 0.01	
	33 (2)		0.30 ± 0.03	< 0.001
	33 (8)		0.24 ± 0.02	< 0.001
	33 (7)	3 days	0.19 ± 0.01	> 0.5
	33 (9)	1 wk	0.18 ± 0.04	> 0.5
H3A + TPA (0.1 g/ml)	39 (4)		0.24 ± 0.02	< 0.001
	33 (2)		0.27 ± 0.01	< 0.001
H3*	39		0.23 ± 0.01	
	33 (4)		0.21 ± 0.01	

[a] The number of chromosomes in different metaphase spreads was found to vary. Therefore, the results are expressed as the mean SCE ± S.E. observed in 20 to 25 metaphase spreads divided by the mean chromosome number.
[b] The probability that these values represent chance variations from that of the control at 39° was determined by Student's t test.
[c] Numbers in parentheses, weeks of exposure.

of integrated PY DNA sequences (4) and increased frequency of SCE. H3A cells were therefore grown at 33° and 39° in the presence of the tumor promoter TPA, which has been reported
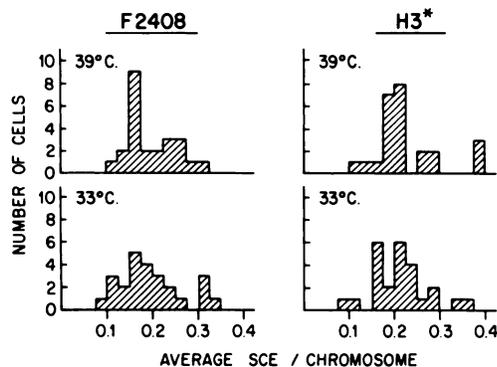
Chart 3. Frequency of SCE in F2408 and H3* cells after growth at 39° and 33° for 6 weeks and 4 weeks, respectively.

to increase SCE frequency (9, 11). The TPA caused an increase in SCE at 39° (Table 1). However, the effects of TPA and T-antigen on SCE were not additive at 33°. As shown by Southern (16) blot hybridization of EcoRI- or BglII-digested DNA to a ^{32}P-labeled PY probe, growth in the presence of TPA did not cause amplification at 39° while at 33° amplification proceeded at the same rate in the presence or absence of TPA (data not shown). These results suggest that there is no simple causal relationship between increased SCE frequency and amplification.

DISCUSSION

We have found an increased spontaneous frequency of SCE in 2 rat cell lines transformed by wild-type PY virus relative to untransformed cells. This result agrees with 2 earlier reports showing an increased level of SCE in cells transformed by SV40 (11) and Rauscher leukemia virus (3). In addition, several cell lines transformed by the ts-a mutant of PY virus displayed a gradual increase in the level of SCE when grown under conditions permissive for large T-antigen function (33°). This increase in frequency of SCE is reversed when cells grown at 33° are shifted back to 39° (nonpermissive for large T-antigen function). One possible explanation for these results is that the growth at 33° (rather than PY T-antigen) is responsible for the observed increase in SCE. However, untransformed rat F2408 cells and a ts-a-transformed cell line which makes a defective large T-antigen fail to exhibit increased SCE when grown at 33°. It is also unlikely that these results are due to selection of cells with elevated SCE levels at 33° since the phenomenon is reversible.

The above results lead us to conclude that a functional large T-antigen is responsible for the observed increase in SCE. However, we are unable to determine at present if this viral product is directly responsible for the increase in SCE. It is possible that SCE may increase as a result of other phenotypic changes in the cell caused by large T-antigen. Certainly the relatively slow increase in SCE frequency when the cells are grown at 33° suggest such an indirect effect of this viral protein.

The ts-a cell lines studied in this report have previously been shown to amplify integrated viral DNA sequences when grown at the temperature permissive for large T-antigen (4). It is possible that unequal crossing-over within the viral DNA sequences induced by large T-antigen might be responsible for

the observed amplification. However, we found that, although the tumor promoter TPA caused an increase in SCE at 39°, it did not induce amplification in H3A cells. Therefore, an increase in SCE frequency at a gross level does not necessarily coincide with amplification of viral sequences. However, it has been suggested that SCE may represent more than one type of phenomenon (15). Therefore, T-antigen and TPA-induced SCE may represent different types of lesions, and the possibility that T-antigen may induce SCE at specific target sites in the DNA cannot be ruled out. It can be ruled out, however, that the increase in SCE is the result, rather than the cause, of the amplification of integrated viral DNA sequences. SCE increase has been observed also in cell lines which contain less than single-copy insertions of viral DNA and do not undergo detectable amplification in culture[3] (data not shown).

If the presence of SCE is considered as a cytological indication of cellular recombination processes, these findings suggest that polyoma large T-antigen acts directly or indirectly to increase recombination. A similar effect of PY large T-antigen may be responsible for its ability to promote the efficiency of cell transformation by PY DNA (6). The data presented here show that, even if a high rate of SCE does not appear to be directly responsible for the amplification of integrated viral DNA sequences, a specific gene product (i.e., large T-antigen) can increase the frequency of SCE.

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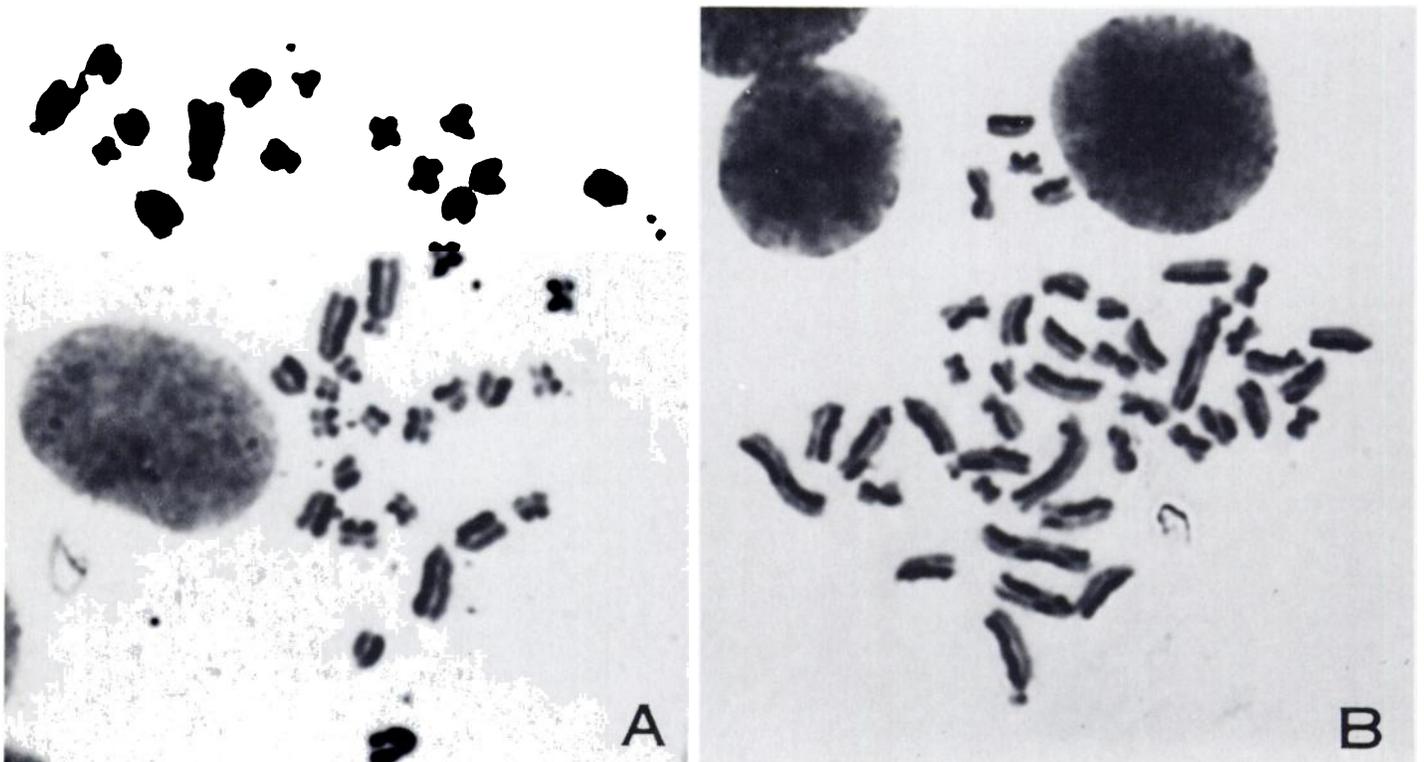
Fig. 1. Micrographs of metaphase spreads illustrating SCE in H3A cells grown at 39° (*A*) or for 2 weeks at 33° (*B*). Spreads were prepared and stained as described in "Materials and Methods" (bromodeoxyuridine, Hoeschst Dye 33258, and Giemsa). × 400.

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