

INTERACTION BETWEEN LATENT PAPOVAVIRUS GENOMES AND THE TUMOR PROMOTER TPA

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1. Introduction

The tumor promoting agent 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) enhances both the transformation of rat embryo cells by adenovirus and the cloning efficiency of such cells [1]. Induction of Epstein-Barr virus (EBV) antigens and stimulation of particle synthesis by treatment of the EBV-carrying cell lines with TPA was reported in [2,3]. Here we report that TPA exclusively enhances the viral DNA content of cells which harbor papovavirus genomes in a latent episomal state. On the other hand, no effect is exerted by TPA on the copy number of the viral DNA in productively infected cells and on integrated papovavirus DNA in transformed cells.

2. Materials and methods

The HD strain of stump-tailed macaque virus (STMV) [4] is produced in Vero cells of monkey origin designated Vero 76. These producer cells arose spontaneously from uncloned Vero 72 cells which carry the HD genomes in a latent state and which do not produce viral capsid antigens. Clonal producer lines such as Vero 76 clone A were established from colonies which grew in softagar [5]. Clonal lines carrying HD genomes in a latent state were obtained by infection of Vero cells with lysates from Vero 76 cells and isolation of colonies under low serum. These cells which were re-cloned twice do not express viral capsid antigens and harbor the viral DNA in an unintegrated episomal state as shown by the Southern technique and by isolation of subclonal lines which have lost the viral DNA [6]. The SV40-transformed GMK-EVa cells were obtained from the Wistar Institute, Philadelphia.

Bovine papilloma type 1 virus (BPV-1) infected mouse embryo fibroblasts contain only unintegrated viral genomes as do naturally occurring equine sarcoid tumors which are induced by BPV-1 [7].

DNA-DNA reassociation kinetic experiments were performed with nick-translated ³²P-labeled HD DNA as a probe. The number of genome equivalents/cell was calculated by comparing the slopes of the curves obtained from reconstruction experiments with the slopes of the experimental curves.

TPA was administered to the cells 3 h after seeding at 55 ng/ml unless otherwise stated. The cells were treated for 5–7 days and then harvested for DNA isolation as in [6]. Blotting was done according to [8].

3. Results

The genomes of a small number of DNA viruses which mainly belong to the herpes-virus group such as EBV [9] and cytomegalovirus [10] can persist in the absence of virion production in a latent state. In case of EBV the DNA can assume the state of a free unintegrated episome [11]. We have reported that the HD strain of STMV behaves similarly and persists in a number of cell lines as an episome [6].

To examine whether the tumor-promoting agent has an enhancing effect on such latent HD genomes as in the case of EBV [2,3] we have compared the viral DNA content of a number of HD containing cell lines with and without treatment with TPA. Examples of reassociation kinetic experiments are shown in fig. 1. Nick-translated ³²P-labeled HD DNA was reassociated either in the presence of herring sperm DNA (○) or, in addition in the presence of increasing amounts of unlabeled HD DNA corresponding either to 0.5, 1 or

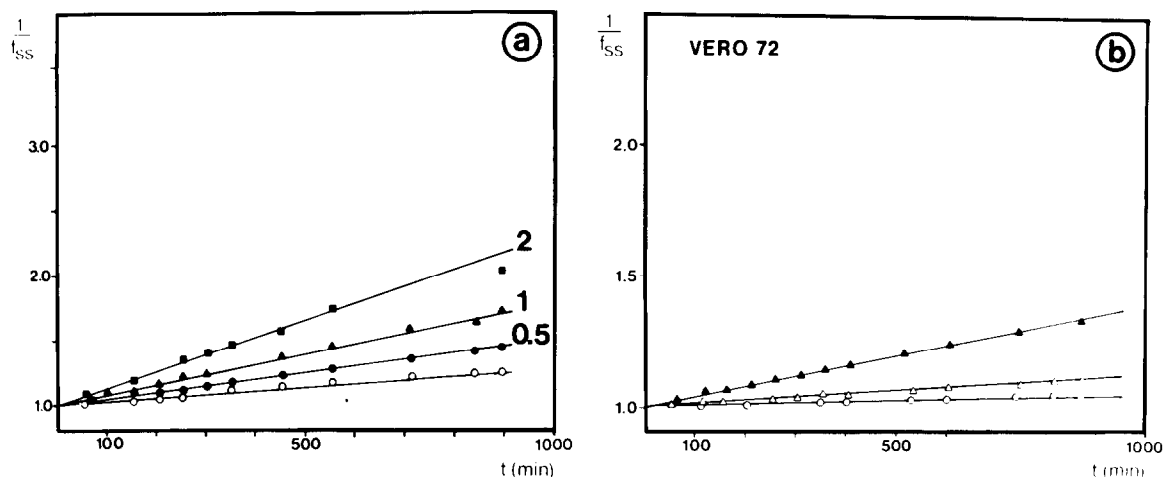


Fig.1. Determination of the HD papovavirus DNA content of non-producer cells with and without TPA treatment by DNA reassociation kinetic experiments. (a) Reconstruction experiments where the reassociation of ^{32}P -labeled HD DNA (10^8 cpm/ μg) was driven in the presence of 2 mg herring sperm DNA/ml (○) and, in addition with unlabeled HD DNA corresponding to 0.5, 1 and 2 genome equivalents/cell as indicated in the figure. (b) Vero 72 cell DNA obtained from untreated (△) and TPA-treated cells (▲) was employed to drive the reassociation of ^{32}P -labeled HD DNA. Circular symbols (○) as in fig.1a. Each point in fig.1 is based upon 2×10^3 cpm which were assayed on hydroxyapatite to determine the double-stranded moiety within the reassociation mixture by chromatography. The data are plotted as fraction of double-stranded DNA against time.

2 genome equivalents/cell (fig.1a). By comparing the slopes of these reconstruction curves with the slopes of the curves in fig.1b one can estimate the relative amount of HD DNA sequences present in HD carrying non-producer Vero 72 cells (without (△) and with TPA treatment (▲)). While untreated Vero 72 cultures contain on the average as little as 0.12 genome equivalents/cell, the TPA treatment amplifies the HD DNA content 6-fold. The relative amount of HD DNA within the non-producer lines is well below 1 genome equivalent/cell because only a fraction of the cell population carries viral episomal DNA. It is increased in all clonal non-producer lines and in the uncloned Vero 72 cells by TPA treatment, although the degree of enhancement varies from clone to clone between 1.3–6-fold (table 1). Increased TPA concentrations (up to 855 ng/ml which are not enlisted in table 1) led to a similar enhancement as did 55 ng/ml.

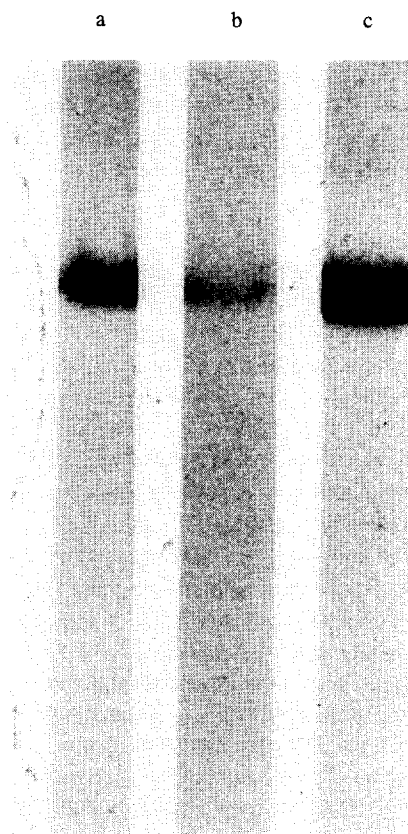


Fig.2. Effect of TPA on bovine papilloma virus DNA replication in mouse embryo fibroblast cells. Four days after infection the cells were passaged (1:4) and TPA was added to 1 culture. After 6 days the DNA was extracted and analyzed by the Southern blot method. (a) To 5 μg cell DNA, 10 genome equivalents of BPV-1 were added; (b) DNA from untreated cells; (c) DNA from cells treated with TPA.

Table 1
Effect of TPA on the viral DNA content in various cell lines

Source of DNA ^a	TPA (ng/ml growth medium)	DNA (mg/ml reassociation mixture)	$\Delta s \times 10^{-3b}$ [min ⁻¹]	Genome equivalents ^c /cell	Enhancing effect of TPA
Non-producer					
Clone II c	0	1	0.07	0.3	
	55	1	0.24	1.0	3.4 ×
Clone IV	0	1	0.07	0.3	
	55	1	0.10	0.4	1.4 ×
Clone V	0	2	0.18	0.4	
	55	2	0.50	1.0	2.8 ×
Vero 72	0	2	0.05	0.1	
	55	2	0.30	0.6	6 ×
Producer					
Vero 76 clone A	0	0.002	0.51	10 × 10 ³	
	10	0.002	0.45	8.9 × 10 ³	
	42	0.002	0.21	4.8 × 10 ³	n.d. ^d
	84	0.002	0.36	7.2 × 10 ³	
	672	0.002	0.34	6.8 × 10 ³	
SV40-transformed lines					
GMK-EVa	0	2	0.77	2.0 ^e	
	55	2	0.86	2.2	1.1 ×

^a Clonal non-producer lines designated IIc, IV and V were established as in section 2. Vero 76 CI A is a clonal HD producer line isolated from soft agar

^b Δs : increase of the slope of the regression curve after addition of the test DNA to the reassociation mixture

^c As determined from reconstruction experiments where 1 ng HD DNA/ml corresponding to 1.14 genome equivalents/cell revealed a Δs of 0.22×10^{-3} [min⁻¹] at a total DNA level of 1 mg/ml. For details see fig.1

^d n.d., not detectable

^e As determined from reconstruction experiments where 1 ng SV40-DNA/ml corresponding to 1.14 genome equivalents/cell revealed a Δs of 0.195 [min⁻¹] at a total DNA level of 1 mg/ml

In contrast, the viral DNA content of the HD-producing Vero 76 clone A cannot be amplified by treatment with the drug even when it is administered to the medium at higher concentrations. Likewise, attempts to stimulate with TPA viral DNA synthesis in cells productively infected with SV40 have failed (unpublished). It is also interesting to note the lack of enhancement in SV40-transformed GMK-EVa cells (and, in SV40-transformed clonal hamster cells (unpublished)) which contain the viral DNA in an integrated state. A most pronounced effect of TPA is shown in fig.2. Mouse cells are non-permissive for BPV-1. When infected, they fail to produce virus but contain non-replicating BPV-1 episomal DNA (fig.2b), which, after a few passages, is lost from the cells.

However, upon TPA treatment we have repeatedly noticed that the BPV-1 DNA content of the cells is increased many-fold probably owing to initiation of replication (fig.2c).

4. Discussion

TPA has a stimulating effect on the viral DNA content of episomal papovavirus DNA carrying non-producer lines. No influence was observed on the expression of viral capsid antigens which remained repressed despite TPA treatment (unpublished). Our results suggest that the physical state of the papovavirus DNA either permits or precludes the stimulating effect: no

enhancement was noticed both in the case of productively infected and of SV40-transformed cells. On the other hand TPA increased the viral DNA content of the non-producer cells which contain either HD or BPV-1 DNA in an episomal state. The mechanism accounting for the observed influence of TPA on episomal papovavirus genomes remains unknown. Their replication may be subjected to cellular control mechanisms that are altered by the tumor promoting agent. Conditions favorable for replication of the episomes might subsequently arise.

Acknowledgements

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