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Variable expression of SV40 large T antigen in CV1 cell clones

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Using immunofluorescence and immunoadsorption, CV1 cell clones MA2, V4, USA3, TR7 and P3 infected with SV40 were found to express variably SV40 large T antigen. The monoclonal antibody used was Pab 419. The results indicate that P3 cells express T antigen to a considerable level as early as 10 h post-infection, while that of TR7 and USA3 cells is minute as judged from their positive nuclei. MA2 and V4 cells did not show any positive nuclei over this period of infection. At 20 h post-infection MA2, V4 and USA3 cells developed a considerable amount of fluorescence in their nuclei while TR7 and P3 cells produced high values. By immunoadsorption of cell extracts for the same periods of infection, similar results were obtained on the electrophoretograms. We also relate these findings with those from induction of heat-shock proteins by SV40 infection.

Cell clone; SV40 T antigen; Gene expression

1. INTRODUCTION

Expression of heat-shock proteins has been found to vary significantly under non-inducing [1] or inducing [2] conditions. This variable expression of heat-shock genes has been postulated as the result of variation in the degree of control of gene expression that depends on cell type [1] or variable differentiation of cloned cells [2].

One of the factors that induce the expression of heat-shock genes is the oncogenic viruses [3–8] which induce these genes during various periods of their infective cycle. Thus, in adenovirus and herpes simplex virus infections, induction of the heat-shock proteins occurs early in infection [5,6] whereas for polyoma and SV40 infections it takes place during the switch from early to late viral functions [7].

We have shown previously that the major heat-shock proteins in CV1 cell clones were variably induced upon thermal treatment [2]. By analogy, it would be of value to investigate whether the same CV1 clones upon SV40 infection express variably the early SV40 gene products. Here, we report variable time-dependent expression of SV40 large T antigen in infected CV1 clones. The heat-shock proteins found in the same clones after viral infection are also discussed.

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2. MATERIALS AND METHODS

2.1. *Cell cultures and viral infection*

Subconfluent cultures (4×10^6 cells per 100 mm inner diameter glass petri dishes) of CV1 clones MA2, V4, USA3, TR7 and P3 were infected with 50 pfu/cell of wild-type SV40, fed with DMEM supplemented with 5% newborn calf serum and incubated at 37°C for the designated periods.

2.2. *Labelling of cultures*

Cultures that were used for immunoadsorption with anti-SV40 large T antigen were labelled for the entire infective period with [^{35}S]methionine (Amersham, spec. act. > 1000 Ci/mmol) in low-methionine DMEM (lm-DMEM) supplemented with 5% NBCS. Control cultures were labelled with 10 μCi, the amounts for 10, 16, 20 and 27 h SV40-infected cultures being 10, 8, 6 and 5 μCi [^{35}S]methionine in 5 ml lm-DMEM per petri dish, respectively.

2.3. *Immunoadsorption of SV40 large T antigen*

The total amount of SV40 large T antigen expressed in CV1 clones at 10, 16, 20 and 27 h post-infection was evaluated by

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precipitating large T antigen with the monoclonal antibody Pab 419 [9]. Large T antigen was extracted in high-pH buffer [10] for 10 min at 4°C. Cell lysates were clarified by centrifugation and the supernatants were used for T antigen precipitation as described by Platt et al. [11] but using high-pH buffer [10]. Pansorbin (Calbiochem, La Jolla, CA) immunoadsorbed SV40 large T antigen was electrophoresed on 10% acrylamide separating gels according to Laemmli [12]. The same amount of acid-precipitable radioactivity from lysates of the various CV1 clones was immunocomplexed, over the various infective periods, and finally applied in each gel slot.

2.4. *Immunofluorescence*

Detection of SV40 large T antigen in situ in infected CV1 clones was performed using the Pab 419 monoclonal anti-SV40 large T antigen counterstained with a 1:20 dilution of fluorescent rabbit anti-mouse IgG (Pasteur Production, Paris). Infected cultures were washed with Dulbecco's phosphate-buffered saline (PBS), pH 7.2, and fixed with 7:3 acetone/methanol for 5 min at −20°C. Fixed monolayers were air-dried and rehydrated with PBS before application of Pab 419. After 30 min at 37°C in a humid chamber, fluorescent rabbit anti-mouse IgG was also applied for another 30 min at 37°C, in a humid chamber. Monolayers were extensively washed with PBS before and after application of antibodies. They were then mounted with 90% glycerol in PBS (pH 10.0), followed by being observed and photographed with epifluorescence on an Olympus microscope.

3. RESULTS

CV1 cell clones MA2, V4, USA3, TR7 and P3 have been shown to possess discrete morphological differences as well as differences in their SDS-PAGE protein profiles [2]. Upon heat treatment these five CV1 clones were found to express variably the major heat-shock proteins. It has been documented that oncogenic viruses induce heat-shock proteins in their permissive cells. Thus, SV40 induces heat-shock proteins in CV1 cells [7]. In order to study this mechanism, we investigated the response of the above CV1 clones towards SV40 infection. We therefore initially performed an immunofluorescence study of the presence of SV40 large T antigen in the nuclei of CV1 cells as a measure of the progression of viral infection followed by determination of the total amount of large T antigen produced by immunoadsorption of long-range radiolabelled large T antigen.

3.1. *Immunofluorescence*

Monolayers of CV1 clones were infected with 50 pfu/cell of SV40. After various periods of infection, cultures were fixed and stained through the indirect immunofluorescence method using the monoclonal antibody Pab 419 (see section 2). The variable expression of SV40 large T antigen in the various CV1 cell clones over different periods of infection is shown in fig.1. Cells from MA2 and V4 clones at 10 h post-infection do not possess large T antigen in any quantity in their nuclei (fig.1;IA,B), those from USA3 and TR7 clones having a minute amount (fig.1,IC,D). However, as early as 10 h post-infection, cells from the P3 clone appear with a considerable amount of large T antigen in their nuclei (fig.1,IE). At 16 h post-infection, cells from the MA2 and V4 clones appear with almost equal amounts of large T antigen in their nuclei (fig.1,IIA,B) to cells of the P3 clone at 10 h post-infection. Meanwhile, cells from USA3 and TR7 clones develop higher levels of fluorescence than MA2 and V4 clones, indicating the presence of greater amounts of T antigen in their nuclei (fig.1,IIC,D). By 16 h post-infection, cells from the P3 clone present a large amount of T antigen (fig.1,IIE). At 20 h post-infection, MA2, V4 and USA3 cells exhibit almost the same level of fluorescence (fig.1,IIIA–C) while TR7 (fig.1,IIID) and particularly P3 cells (fig.1,IIIE) develop still higher levels. Advancing the observations to as late a stage as 27 h post-infection, well into the late phase of the SV40 lytic cycle, almost all P3 and TR7 cell nuclei are positive for SV40 large T antigen, while USA3, V4 and MA2 cells still appear with negative nuclei (not shown). Observations made at 6 h post-infection showed a faint positive reaction taking place in P3 cells only (not shown). Carrying out the same observations with synchronous cultures of CV1 clones, all five clones gave the same results with respect to the presence of large T antigen in their nuclei, but with a delay of almost 4 h.

3.2. *Immunoadsorption*

We also used the specific immunoadsorption procedure of Platt et al. [11] to investigate the total amount of SV40 large T antigen expressed in the same cell clones and over the same periods of infection. Large T antigen was first complexed with the Pab 419 antibody, followed by adsorption of the immunocomplex onto pansorbin cells. The various cultures were labelled over the entire infective period. Fig.2 depicts the electrophoretograms of the specific immunoadsorbed large T antigen. Although with this procedure the actual amount

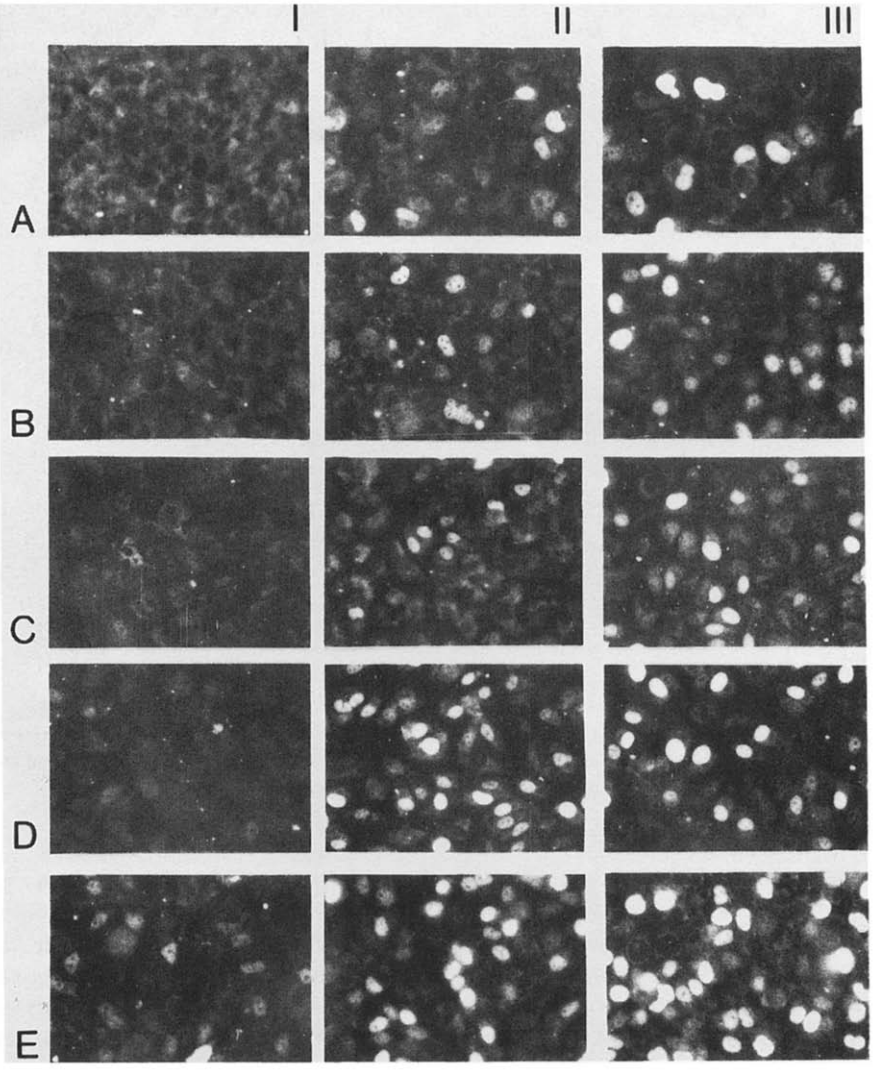

Fig.1. In situ detection of SV40 large T antigen synthesized in the various CV1 clones. (A) MA2, (B) V4, (C) USA3, (D) TR7, (E) P3 cells. All cells were infected with SV40 for: (I) 10 h, (II) 16 h, (III) 20 h.

originally immunocomplexed onto pansorbin cells is reduced [11], our observations verify that the above technique is quantitatively and qualitatively reproducible. Fig.2 indicates that the various clones express the SV40 large T antigen to different levels. P3 cells express the large T antigen to a high level (fig.2,E4,5), with TR7 cells (fig.2,D4,5) producing a comparable value. On the other hand, MA2, V4 and USA3 cells express T antigen to a lesser degree (fig.2A,B,C4,5, respectively). Following the non-specific adsorption of large T antigen-Pab 419 immunocomplex onto pansorbin cells, i.e. the pre-SDS step of Platt et al. [11], the protein profiles obtained are comparable but with additional protein bands that do not correspond to the electrophoretic mobility of T antigen (unpublished). At 16, 20 and 27 h post-infection, the amount of large T antigen extracted from the various CV1 clones (fig.2) is comparable to the fluorescence level observed in the respective cells (fig.1). Several bands that appear very faint on the electrophoretograms do not represent T antigen

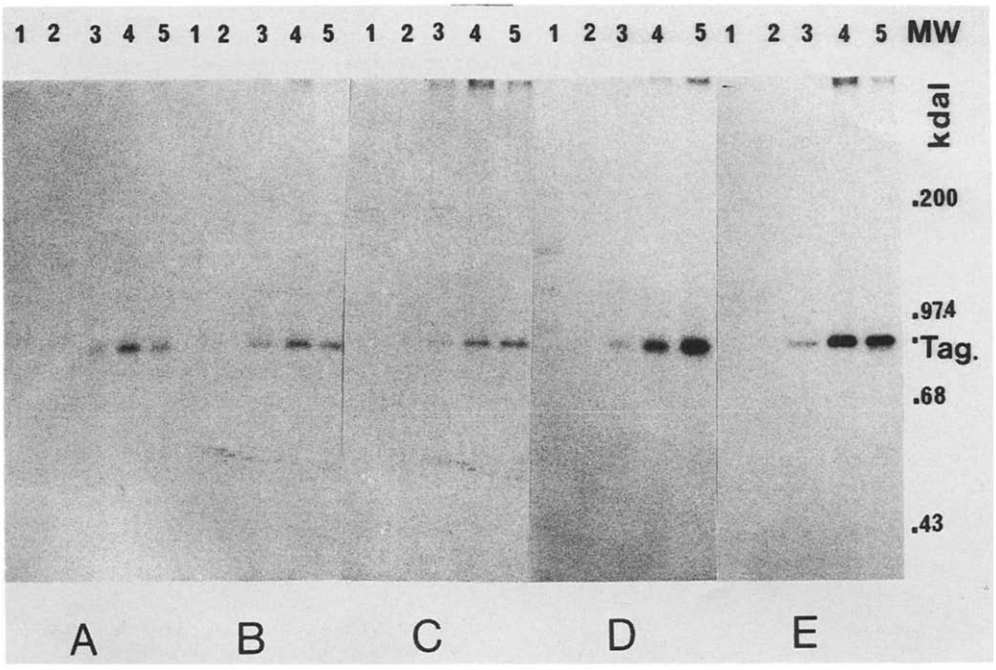

Fig.2. Electrophoretograms of immunoadsorbed SV40 large T antigen from the various CV1 clones. Immunocomplexes were separated on 10% acrylamide gels. (A) MA2, (B) V4, (C) USA3, (D) TR7, (E) P3 cells. All cells were infected with SV40 for: (1) 0 h, (2) 6 h, (3) 16 h, (4) 20 h, (5) 27 h. Tag., SV40 large T antigen. Molecular mass standards: myosin heavy chain (200 kDa), phosphorylase *b* (97.4 kDa), bovine serum albumin (68 kDa), egg albumin (43 kDa).

forms as they appear on the control lanes as well. This distinction becomes clearer on inspecting the electrophoretograms of the non-specific immunoadsorption procedure. When equal volumes of lysates were immunocomplexed onto pansorbin cells instead of the same amount of acid-precipitable radioactivity, almost the same electrophoretograms were obtained.

4. DISCUSSION

SV40 large T antigen is a non-structural viral protein which is responsible for several regulatory activities, such as ATPase [13], specific binding to SV40 DNA [14] and non-specific binding to DNA [14,15]. The above-mentioned activities can be assigned to distinct domains of the polypeptide chain. The majority of the large T antigen molecules are converted by post-transcriptional modification into oligomers [16–20] and this may play a role in viral but not in cellular DNA replication [21]. T antigen also forms stable complexes with the host protein p53 in a species-dependent way [17,22–24]. The present results suggest that as the rate of synthesis of T antigen varies in different CV1 clones, variable processing that would result in altered activities should be expected. Other experiments (to be published elsewhere) indicate that this differential expression of T antigen reflects variable SV40 mRNA synthesis in the different CV1 clones. It was reported that the sedimentation profiles of SV40 large T antigen were identical in several SV40-infected monkey cell lines [25]. This may be so as the sedimentation properties of T antigen were studied very late in infection. In contrast, our results derived from cells that were in the early phase of viral infection as well as in the transition period from early to late SV40 gene expression.

If we consider that the induction of heat-shock proteins in the infected cells is a direct or indirect activity of the early SV40 gene products [7], then variable induction of heat-shock proteins in the various CV1 clones must be expected. We actually

observed this variable expression of heat-shock proteins (unpublished). The cell clone that expressed SV40 large T antigen (P3) to the highest degree appeared with the least induction of heat-shock proteins. As pointed out in an earlier publication [2], upon thermal treatment P3 cells induced the 70 kDa major heat-shock protein to the highest level.

Imperiale et al. [1], using another virus-cell system, reported that cell transcriptional factors may be responsible for expression of the 70 kDa major heat-shock protein under non-inducing conditions. Our results suggest that such transcriptional factors may exist but their mode of action cannot be elucidated; we can only suggest that in our system, they show a greater affinity towards early SV40 than heat-shock genes.

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