Changes in nuclear distribution of large tumor antigen correlate with the proliferative activity of simian virus 40-transformed cells

GAMALIEL E. ORDENES, INGRID BECK, MARIA T. CARMONA and MYRIAM SANTOS*

Departamento de Biologia Celular y Genetica, Facultad de Medicina, Universidad de Chile, Casilla 70061-Santiago 7, Chile

* Author for correspondence

Summary

A correlation between intranuclear distribution of large tumor antigen (T-ag) and proliferative activity in Simian virus 40 (SV40)-transformed cells has been established. Nuclear T-ag was cytochemically detected by the immunoperoxidase reaction. Cell populations with a high growth rate displayed a heterogeneous pattern with patches of positive reaction surrounded by large and irregular negative areas. By contrast, cell populations with a low growth rate presented a rather homogeneous distribution of nuclear T-ag with a finely granular positive reaction all over the nucleus, excluding nucleolus-like areas. The latter pattern was also observed in cells that had been inhibited from traversing S phase. In this case, the heterogeneous distribution of T-ag within the nucleus was recovered once the cells were allowed to proceed again through S phase, or through G_2 phase. Highly condensed chromatin in mitotic chromosomes appeared to exclude T-ag fully as early as in prophase. Then, nuclear distribution of T-ag changes throughout the cell cycle, thus suggesting it might be a cell cycle-dependent event. Interestingly, thymidine-labeled cells from cultures with a high growth rate presented a heterogeneous distribution of autoradiographic grains. Regardless of their mechanism(s) of origin, T-ag-enriched areas might correspond to nuclear sites where a specific function, such as DNA synthesis, is being carried out.

Key words: cell growth, SV40-transformed cells, nuclear T-antigen.

Introduction

Intracellular redistribution of a particular macromolecule usually reflects important changes in its functional state. A number of nuclear proteins, such as cyclin (Celis & Celis, 1985; Kurki *et al.* 1986; Madsen & Celis, 1985), small nuclear ribonucleoprotein particles (U-snRNPs; Ringertz *et al.* 1986; Spector & Smith, 1986), lamins (Bludau *et al.* 1986; Chaly *et al.* 1984; Gerace *et al.* 1978) and others (Bhorjee *et al.* 1983; Todorov *et al.* 1988), have been shown to be located in particular areas within the nucleus, which change as cells progress through the proliferative cycle. Thus, it has become increasingly important to determine changes in the subcellular distribution of a given protein, as an approach to understanding its function.

Simian virus 40-transformed mouse cells synthesize the virus-coded large tumor antigen (T-ag), which is a multifunctional protein largely concentrated in the nucleus (for a review, see Rigby & Lane, 1983). Among other functions, T-ag has the ability to induce cellular DNA synthesis (Galanti *et al.* 1981; Graessman & Graessman, 1976; Soprano *et al.* 1983; Tjian *et al.* 1978), and it might actively participate in such a process because of its cellular DNA-binding property (for a review, see Rigby & Lane, 1983) and ATP-dependent DNA helicase activity (Stahl *et al.* 1986).

Staufenbiel & Deppert (1983) have shown that in asynchronously growing SV40-transformed cells most of the nuclear T-ag is at the nucleoplasm, while a minor fraction of it appears to be associated with either nuclear matrix or chromatin. If T-ag is actively involved in cellular DNA synthesis it is reasonable to suspect that those subpopulations of nuclear T-ag would be quantitatively modified in relation to the proliferative state of the cells, thus giving rise to variations in the intranuclear distribution of this viral protein.

These considerations led us to investigate whether the location of T-ag within the nucleus changes according to the cell growth rate. To this end, the nuclear distribution of T-ag was cytochemically detected in cell cultures with different proliferative activities. In this study we have found that a distinct heterogeneous pattern of intranuclear T-ag distribution appears to be related to the proliferative activity, and it is mainly displayed by cells traversing both S phase and G_2 phase. Since chromatin

condensation seems to exclude T-ag, nuclear distribution of this protein might be the passive result of such an event taking place to a different extent throughout the cell cycle. Regardless of the mechanism(s) that may determine T-ag location, the possibility exists that T-ag-enriched areas might represent nuclear domains where T-ag is participating in some specific function, such as DNA synthesis.

Materials and methods

Cell lines

The transplantable mKSA-Asc line of SV40-transformed Balb/c kidney cells (Kit *et al.* 1969), the WTB1a line of wild-type SV40-transformed Balb/c 3T3 cells (Brockman, 1978) and the A255B1b line of Balb/c 3T3 cells transformed by the SV40 temperature-sensitive mutant tsA255 (Brockman, 1978), all of them kindly provided by Dr Janet Butel, were used in this study. Cells were routinely cultured as described (Santos & Butel, 1982).

Effect of hydroxyurea upon cellular distribution throughout the cell cycle

Cells (5×10^5) were seeded in 35 mm tissue culture plates (Falcon, Becton Dickinson Co., Oxnard, CA) in Dulbecco's modified Eagle's Minimal Essential Medium (D-MEM, Grand Island Biological Co., Grand Island, NY) supplemented with 0·5 % fetal bovine serum (FBS, Grand Island Biological Co.) and incubated for 24 h at 37 °C. At this time, medium was replaced by D-MEM supplemented with 10 % FBS, and hydroxyurea (HU; Sigma Chemical Co., St Louis, MO) was added to experimental plates. After 12 h of incubation at 37 °C, cells were washed with warmed Hanks' solution (Grand Island Biological Co.) and fresh D-MEM supplemented with 10 % FBS was added to each plate. The fraction of cells in every phase of the cell cycle was estimated at 2-h intervals by a method that combines microspectrometry, autoradiography and morphology (Mak, 1965).

Immunocytochemical labeling

Nuclear T-ag was detected by a slightly modified peroxidase–antiperoxidase (PAP) reaction (Sternberger *et al.* 1970). Cells grown on coverslips were fixed with cold methanol for 15 min at 4 °C and air dried. Cells were then incubated with 3 % H_2O_2 in distilled water for 5 min at 20 °C, washed three times with phosphate-buffered saline (PBS), and sequentially incubated with a 1/50 dilution of either rabbit anti-T-ag polypeptide antiserum (RAT, Lanford & Butel, 1979) or normal rabbit serum (NRS, negative control), for 30 min at 37 °C; with a 1/100 dilution of goat anti-rabbit immunoglobulin antiserum (GAR; Cappel Laboratories, Cochranville, PA) for 30 min at 20 °C, and with a 1/50 dilution of peroxidase–antiperoxidase complex (PAP; Cappel Laboratories), for 30 min at 20 °C. Cells were washed three times with PBS after each incubation period. Finally, they were incubated for 15 min at 20 °C with a freshly prepared solution of 0·05 % 3,3'-diaminobenzidine (DAB; Sigma Chemical Co.), containing 0·03 % H_2O_2, dehydrated and mounted for light microscopy. Processing for electron microscopy was similar except that cells were fixed in 5 % formaldehyde and permeabilized with ethanol. The reacted cells were incubated with 1 % OsO_4 for 1 h at room temperature, dehydrated and embedded in LX112 resin (Ladd Research Industries, Inc. Burlington, VE) while still attached to the coverslips. Ultrathin sections were examined without further staining.

Radioactive labeling and autoradiography

Cells were pulse-labeled with [^{3}H]thymidine, washed extensively, fixed and processed for autoradiography (Baserga & Malamud, 1969). Radioactive thymidine incorporation into an acid-precipitable material was determined as described (Landford & Butel, 1981).

Results

Nuclear T-ag distribution and cell growth rate

Previous detections of nuclear T-ag by indirect immunofluorescence have shown a punctate positive reaction all over the nucleus except in the nucleoli of SV40-transformed cells (Pope & Rowe, 1964; Rapp *et al.* 1964). This time, nuclear distribution of T-ag has been determined by the peroxidase–antiperoxidase reaction, which is a much more sensitive method than the previous one. In order to test whether specific patterns of reactivity are related to the proliferative state of cells we analyzed the nuclear distribution of T-ag in relation to cell growth rate.

Populations of mostly resting or mostly dividing SV40-transformed mouse cells may be obtained by varying the number of cells that are seeded (Santos & Butel, 1985), or the concentration of FBS in the medium. Thus, by culturing cells as indicated in the legend to Fig. 1, we have defined a cell culture with a high proliferative activity (type A) and another one with a low proliferative activity (type B), on the basis of their rate of [^{3}H]thymidine incorporation into acid-precipitable material as well as with regard to the corresponding labeling and mitotic indices (Table 1). According to these results, the proliferative fraction in type A cultures would be two- to-threefold greater than that observed in type B cultures. These results also indicate that cells from type A cultures present a significantly increased rate of DNA synthesis as compared to those from type B cultures.

When large T-ag was detected by the PAP reaction in asynchronously growing cells, various patterns of reactivity were observed (data not shown). However, when type A cultures were analyzed, most of the cells (85 %) displayed patches of positive reaction surrounded by large and irregular negative areas, which included the nucleoli (Fig. 1A,B), whereas about 80 % of cells in type

Table 1. *Proliferative characteristics of cell cultures*

Type of culture*	Incorporation of [^{3}H]TdR† (cts min^{-1}, $\times 10^{-4}$/ 10^6 cells)	Labeling index‡ (%)	Mitotic index§ (%)
A	14·0	65	20
B	0·3	29	7·6

* Type A and type B cultures were established as described in the legend to Fig. 1.

† Incorporation of [^{3}H]thymidine ([^{3}H]TdR) in trichloroacetic-precipitable material was measured after a 30-min pulse ($n = 6$).

‡ Labeling index was determined, in a total of 1000 cells, after a 30-min pulse with [^{3}H]thymidine followed by autoradiography ($n = 2$).

§ Mitotic index was determined by counting cells traversing mitosis, out of a total of 1000 cells ($n = 2$).

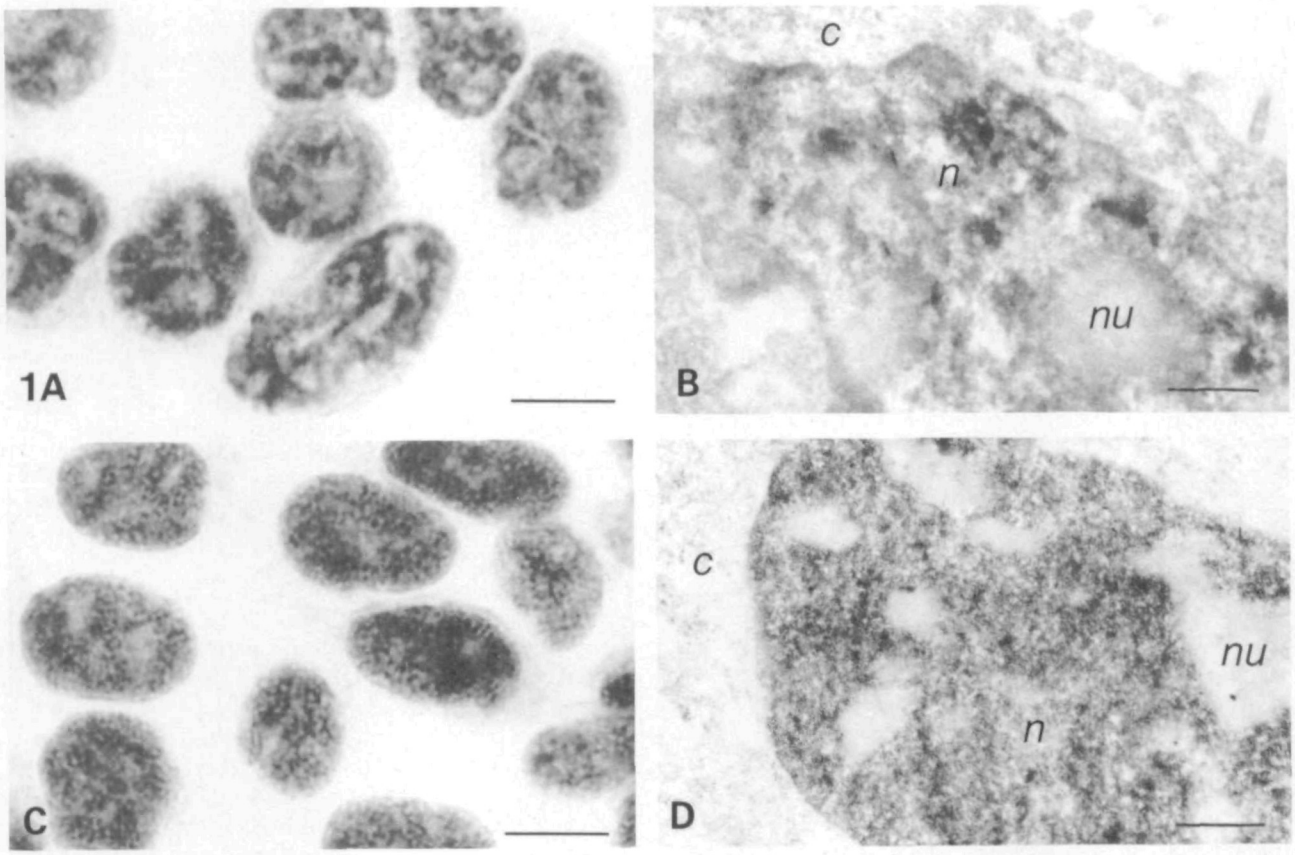

Fig. 1. Nuclear distribution of large tumor antigen in cell cultures with different growth rates. Either 1×10^4 (type A cultures) or 1×10^5 (type B cultures) mKSA-Asc cells cm^{-2} were seeded on coverslips in 35 mm plates and incubated at 37°C for 24 h in D-MEM supplemented with 5% FBS. Type B cultures were further incubated for another 24 h at 37°C in D-MEM supplemented with 0·5% FBS. After the corresponding incubation periods, cells were washed, methanol-fixed, stained by the PAP reaction, and processed for both light and electron microscopy, as described in Materials and methods. A,B. Cells in type A cultures; C,D, cells in type B cultures; A,C, light microscopy; B,D, electron microscopy. *n*, nucleus; *c*, cytoplasm; *nu*, nucleolus. Bars: A,C, 10 μm, ×1400; B,D, 1 μm, ×12 500.

B cultures presented a finely granular positive PAP reaction all over the nucleus, except in nucleolus-like areas (Fig. 1C,D). Such patterns of nuclear T-ag distribution, defined as heterogeneous and homogeneous, respectively, were observed in sections as well as in intact cells. Besides, they were also obtained when the PAP reaction was carried out following cell fixation in either cold ethanol (15 min at 4°C), paraformaldehyde (4% in PBS, 30 min at 4°C) or formaldehyde (4% in PBS, 30 min at 4°C), thus discarding the possibility of an artifactual observation.

Cellular DNA synthesis and nuclear distribution of T-ag

It has been shown above that in type A cultures most of the cells display a heterogeneous pattern of nuclear T-ag distribution. According to data in Table 1, the majority of cells in those cultures are in S phase. Therefore, it was reasonable to postulate that such a distinct pattern of nuclear T-ag might correspond mostly to that in cells that are replicating their DNA.

Interestingly, regardless of the type of culture, a heterogeneous distribution of silver grains was observed when autoradiography was carried out following a 30-min pulse labeling with [^{3}H]thymidine (Fig. 2A). These results showed that nuclear sites where DNA synthesis is taking place are, at a given time, heterogeneously distributed within the nucleus.

As an indirect approach to studying the relationship between nuclear distribution of T-ag and cellular DNA synthesis, cells in equivalent cultures were subjected to either PAP reaction or autoradiography, following a 12-h labeling with [^{3}H]thymidine. This labeling time was selected by considering both the cell cycle time ($t_c = 16{\cdot}3$ h) and the S phase time ($t_s = 7$ h) of mKSA-Asc cells (unpublished results), thus allowing every cell to proceed through S phase at least once during the labeling period. Under these experimental conditions and according to data in Table 1, one would expect labeled cells in type A cultures to correspond to those that are actively synthesizing DNA. By contrast, labeled cells in type B cultures correspond to those that were replicating their DNA at the time the radioactive precursor was added to the medium, but are rather quiescent by the end of the 48-h incubation period. Therefore, silver grains in cells from type A cultures should represent recently

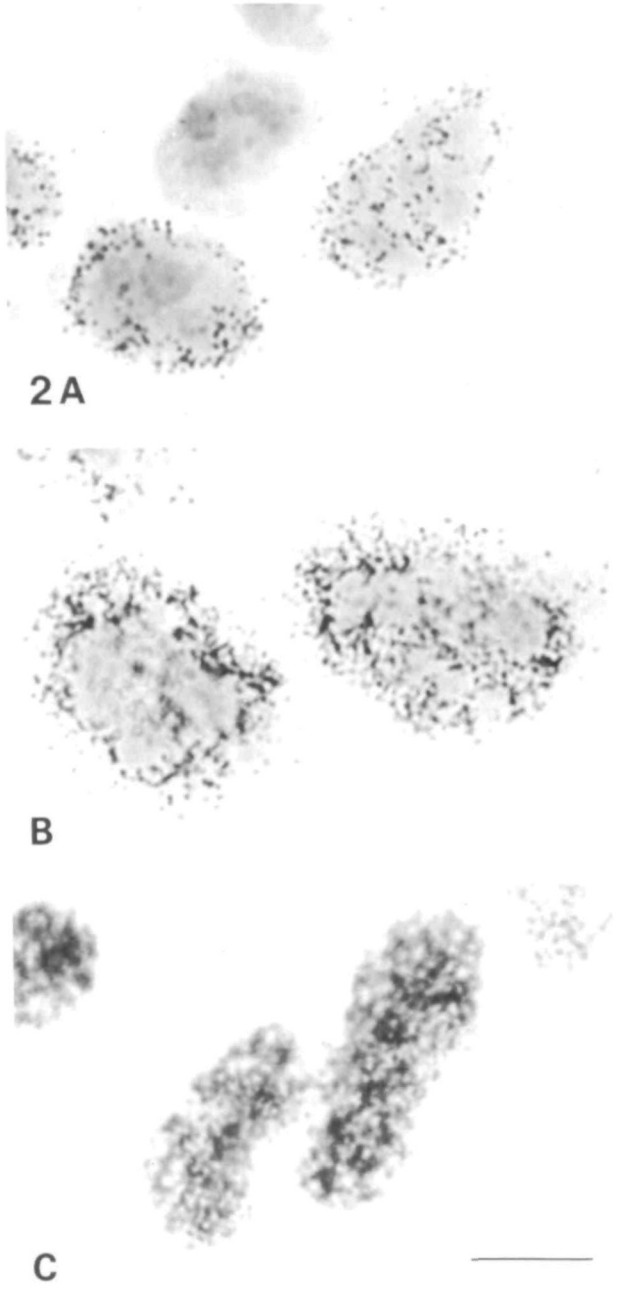

Fig. 2. Cellular DNA synthesis and nuclear distribution of autoradiographic silver grains. mKSA-Asc cells in type A and B cultures were incubated in the presence of [^{3}H]thymidine (0·5 μCi ml^{-1}; 60 Ci mmol^{-1}) during the final 30 min or 12 h of the corresponding culturing periods (24 h and 48 h, respectively). After radioactive labeling, cells were processed for autoradiography and nuclear distribution of silver grains was determined by light microscopy. A. 30-min labeled cells from type A cultures; B, 12-h labeled cells from type A cultures; C, 12-h labeled cells from type B cultures. Bar, 10 μm, ×1600.

synthesized DNA, while silver grains from type B cultures should represent previously synthesized DNA.

The majority of cells in type A cultures displayed a heterogeneous nuclear distribution of silver grains (Fig. 2B), while most cells in type B cultures presented a homogeneous one (Fig. 2C). Thus, an intriguing similarity exists between nuclear location of autoradiographic silver grains and the pattern of nuclear T-ag distribution shown before (Fig. 1A and C, respectively). On the basis of these results, it is tempting to speculate that T-ag-enriched nuclear areas in cells from type A cultures might correspond to nuclear sites where DNA is being synthesized. Certainly, convincing evidence about co-location of T-ag and autoradiographic grains can be only obtained by simultaneous examination of both of them. On the other hand, considering that T-ag is a multifunctional protein, then its homogeneous distribution in those rather quiescent cells from type B cultures, which might be given by nucleoplasmic and/or nuclear matrix-associated T-ag molecules, could be functionally related to any other cellular process(es).

Table 2. *Effect of hydroxyurea on the nuclear distribution of large tumor antigen*

Time after treatment (h)	Cell cycle distribution (%)				Nuclear distribution of large tumor antigen (%)	
	G_1	S	G_2	M	Homogeneous*	Heterogeneous†
0	80	0	20	0	70	30
4	14	75	10	1	40	60
8	9	8	80	3	30	70

The effect of hydroxyurea on both cellular distribution along the cell cycle and nuclear location of large T-ag were determined as described in Materials and methods ($n = 3$).

* Homogeneous: finely granular positive reaction distributed all over the nucleus, with the exception of nucleoli.

† Heterogeneous: patches of positive reaction with large and irregular negative areas.

To investigate whether the heterogeneous pattern of nuclear T-ag distribution is related to DNA replication, we have determined the effect of a temporal inhibition of cellular DNA synthesis by hydroxyurea (HU) upon the location of T-ag within the cell nucleus. To define the effect of HU upon the proliferative state of mKSA-Asc cells, the frequency of cells in each phase of the cell cycle was determined at time zero and at 2-h intervals after treatment. As shown in Table 2, by the end of a 12-h incubation in the presence of 1·0 mM-HU, 80 % of cells were in G_1. No labeled cells were found at this time, but they appeared immediately after drug removal, reaching a maximum of about 75 % by 4 h after the end of the treatment. As expected, by the time the frequency of cells in S phase had decreased to a minimal value (8 h after HU removal) the frequency of cells in G_2 had increased to about 80 %. Therefore, DNA synthesis was effectively inhibited by HU treatment. Importantly, treated cells underwent division about 10 h after blockade suppression, thus showing that they had recovered from the effects of hydroxyurea.

Nuclear T-ag was detected by PAP reaction immediately after a 12-h treatment with HU (time 0) or at 4 h or 8 h following drug removal from the culture medium. The percentage of cells showing either the heterogeneous or homogeneous pattern was determined in both cases

(Table 2). By the end of the HU treatment, when 80 % c cells are supposed to be in the G_1/S transition (unpub lished results, Chang & Baserga, 1977), most of the cell displayed the homogeneous reaction (Table 2). A signif cant increase in the number of cells displaying th heterogeneous pattern of nuclear T-ag was detected b the time most of the cells were in S phase. A mino although repetitive, increase was then observed when th majority of cells were traversing the G_2 phase (Table 2) Therefore, a similar heterogeneous pattern of intran clear distribution of T-ag is displayed by cells in S and G phases of the cell cycle.

According to morphological observations, chromati in G_2 cells appeared to be condensed to some extent (dat not shown). In this respect, it has been known for a lon time (Pope & Rowe, 1964; Rapp *et al.* 1964) that T-ag not detected in the highly condensed chromatin c chromosomes in mitotic cells. Therefore, it is attracti to postulate that chromatin condensation, which migl start as soon as DNA has been replicated, provokes change in nuclear T-ag distribution, thus explaining th fact that most of the cells traversing G_2 phase display heterogeneous distribution of T-ag within the nucleus.

Interestingly, when T-ag was cytochemically detecte in cells traversing mitosis, it was found that its exclusio from condensed chromatin had started early in propha (Fig. 3B), it became evident by metaphase (Fig. 3C) an it remained so throughout anaphase (Fig. 3D) and tel phase (Fig. 3E). A sharp and dark positive reactio appeared, lining the mass of unreactive chromosome especially during telophase (Fig. 3E), just before th nuclei were reconstituted. Whether such a reaction a pears in the pictures or not depends on the focal plan T-ag remained cytoplasmic throughout mitosis and became nuclear again by the end of cell divisio (Fig. 3F). Nuclear distribution of T-ag in the two ne cells, which must be entering G_1 phase (Fig. 3F), w similar to that observed before in type B cell cultur (Fig. 1C,D) and in those cells at the G_1/S bounda (Table 2). The latter results suggest that the hom geneous distribution of nuclear T-ag might be typical of G_1 cells.

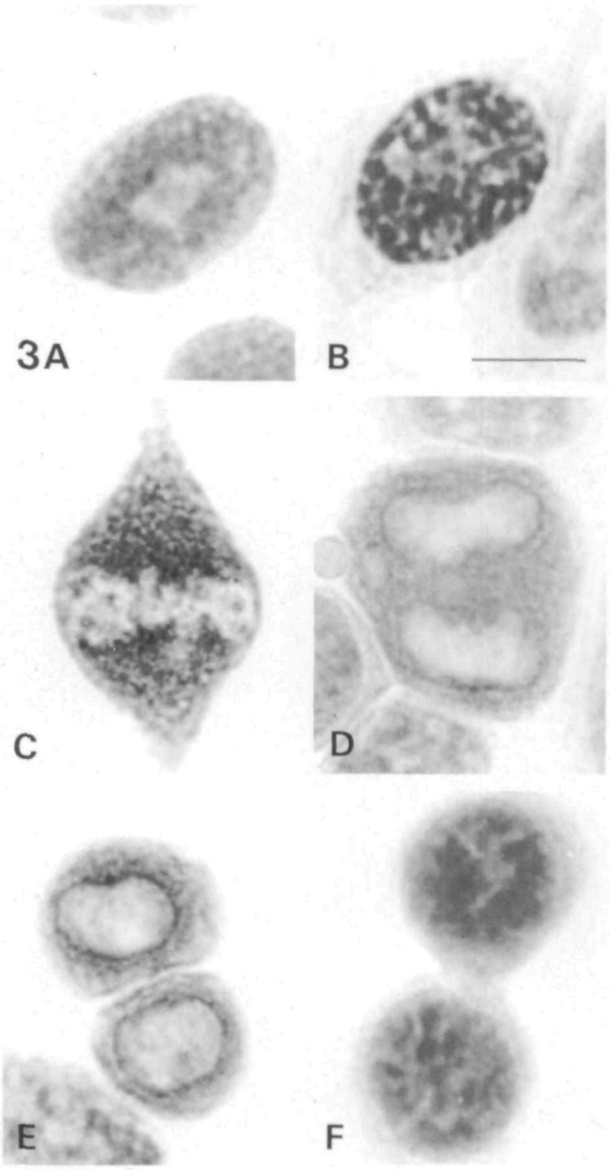

Fig. 3. Intracellular distribution of large tumor antigen throughout mitosis. Cells were seeded on coverslips in 35 mm tissue culture plates, and cultured as routinely. After a 24-h period of incubation, cells were fixed with cold methanol for 15 min and large T-antigen was detected by the PAP reaction. Mitotic cells were morphologically identified. Interphase (A), prophase (B), metaphase (C), anaphase (D), telophase (E), late telophase (F). Bar, 10 μm; ×1500.

Cellular phenotype and nuclear distribution of T-ag

A possibility to be considered is whether the patterns of nuclear T-ag distribution described above are related to the transformed phenotype, rather than to the cell growth rate. To evaluate that possibility, the nuclear distribution of T-ag was analyzed in A255B1b cells, when grown at either permissive (33 °C) or restrictive (39·5 °C) temperatures. Under these two culturing conditions A255B1b cells express either the transformed or the normal phenotype, respectively (Brockman, 1978). As shown in Fig. 4, exponentially growing A255B1b cells displayed a heterogeneous pattern of nuclear T-ag at both permissive (Fig. 4A) and restrictive temperatures (Fig. 4B). A similar result was obtained when WTB1a cells, which express the transformed phenotype in both culturing conditions, were used (Fig. 4E,F).

On the other hand, when confluent monolayers with a low growth rate were used a homogeneous pattern of nuclear T-ag distribution was observed under either permissive or restrictive conditions, in both A255B1b (Fig. 4C,D) and WTB1a (Fig. 4G,H) cell lines. These results strongly suggest that intranuclear distribution of T-ag correlates with cell growth rate rather than with cellular phenotype. They also show that variations in the distribution of T-ag within the nucleus in relation to the proliferative activity is not a cell line-dependent phenomenon, since at least three different SV40-transformed mouse cell lines expressed the same behavior.

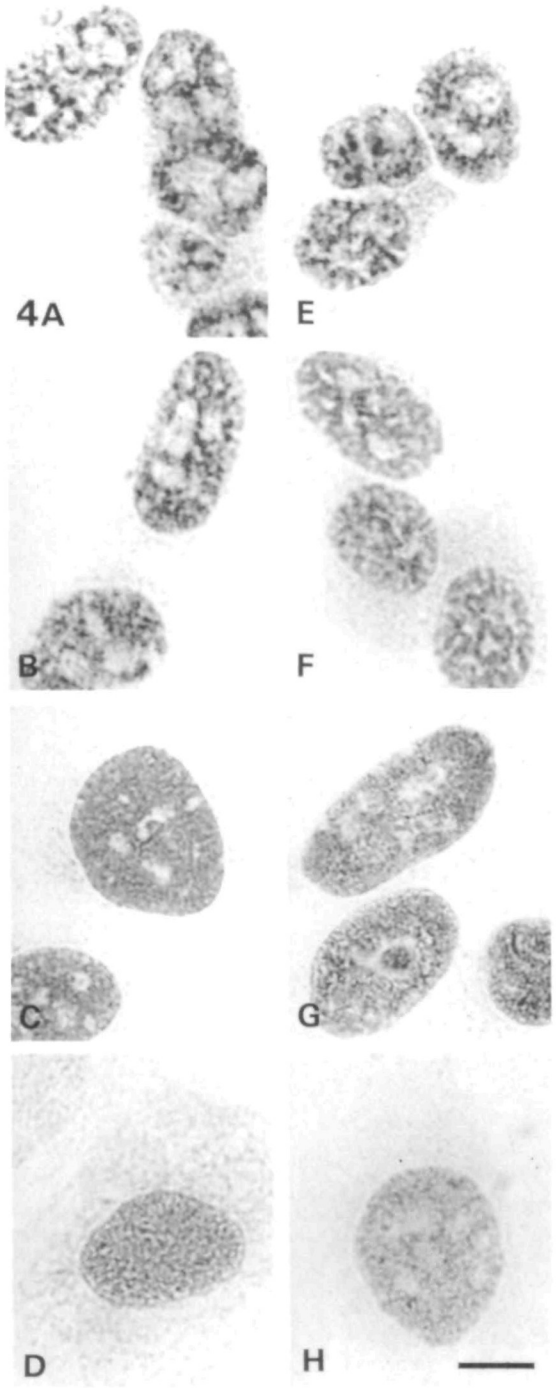

Fig. 4. Cellular phenotype and nuclear T-ag distribution. A255B1b and WTB1a cells were seeded in 60 mm tissue culture plates and incubated at 33 °C for 2 h in D-MEM supplemented with 5 % FBS. At this time, when cells had already attached to the substratum, medium was replaced by D-MEM supplemented with 1 % FBS and they were further incubated for either 24 h or 48 h at 33 °C or 39·5 °C. Nuclear T-ag was detected by the PAP reaction at the end of each incubation period. A–D. A255B1b cells; E–H, WTB1a cells; A,E, exponentially growing cells cultured at 33 °C for 24 h; B,F, exponentially growing cells cultured at 39·5 °C for 24 h; C,G, confluent monolayers of cells cultured at 33 °C for 48 h; D,H, confluent monolayers of cells cultured at 39·5 °C for 48 h. Bar: 10 μm; ×1200.

Discussion

The understanding of the functional and spatial organization of the interphase nucleus has become, in recent years, an intriguing and interesting aspect of cellular physiology. It has been postulated that interphase chromatin might be organized into functional domains, such as those involved in either RNA transcription, RNA processing or DNA replication (for a review, see Ringertz *et al.* 1986). Accordingly, the subnuclear location of any given protein appears to be related to its particular function. Cyclin, for instance, presents a temporal and spatial correlation with cellular DNA synthesis (Celis & Celis, 1985; Bludau *et al.* 1986; Madsen & Celis, 1985), thus raising the possibility that it might be involved in such a process. In this respect, it has been recently shown that cyclin is the auxiliary protein of DNA polymerase-δ, which is required for the elongation stage of DNA synthesis (Bravo *et al.* 1987; Prelich *et al.* 1987; Prelich & Stillman, 1988). Therefore, cyclin-enriched nuclear sites should correspond to replicative domains, which are expected to be modified during the cell cycle.

Data presented here show that nuclear distribution of T-ag varies according to cell growth rate in SV40-transformed mouse cells. While it is homogeneously distributed within the nucleus of resting cells, it appears to be mainly located in nuclear patches in dividing cells. Interestingly, following inhibition of cellular DNA synthesis, cells display the homogeneous pattern of nuclear reactivity. Cells in S phase, instead, present a heterogeneous pattern more like that observed in actively growing cells. These results suggest that nuclear T-ag distribution might be also related to some aspects of cellular DNA synthesis. Such a suggestion is further substantiated by those results that show a similarity between T-ag subnuclear location and nuclear sites where DNA replication is taking place in cells from type A cultures (Fig. 1A and Fig. 2A or B, respectively). However, cells in G_2 phase, which are not synthesizing DNA, also present a heterogeneous pattern of nuclear T-ag. Thus, it may be assumed that, in general, T-ag location within the nucleus might be consequential to its exclusion from condensed chromatin. Nevertheless, it cannot at present be ruled out that T-ag-enriched areas might result from specific interactions of T-ag with other nuclear components. Regardless of the mechanism(s) determining its location, the possibility exists that T-ag may be playing a specific role in those nuclear sites in which it is present at a given time.

Interestingly, SV40 DNA synthesis has become the model of choice for analyzing the molecular basis of eukaryotic DNA replication (for a review, see Stahl & Knippers, 1987). In this system, T-ag has been postulated to have a role in both initiation and elongation stages of SV40 DNA synthesis, according to the replicon model (Campbell, 1988). Since T-ag has a cellular DNA binding property (for a review, see Rigby & Lane, 1983) and an intrinsic helicase activity (Stahl *et al.* 1986), it might also be involved at least in the elongation stage of cellular DNA synthesis, and is probably therefore a component of nuclear matrix-associated replication com-

plexes (Carri *et al.* 1986; Jackson & Cook, 1986). As DNA replication is a highly asynchronous process, one would expect replication complexes to be heterogeneously distributed within the nucleus. Correspondingly, we have found that intranuclear distribution of T-ag is heterogeneous in actively growing cells, thus allowing us to speculate that those areas where T-ag is located might correspond to replicative domains in the nucleus of SV40-transformed mouse cells. In this respect, it is very important to point out the fact that Harper *et al.* (1985), have shown that nuclear sites where SV40 DNA is being synthesized, during the lytic infection, are characterized by an accumulation of T-ag.

Finally, T-ag has been shown to be important for cells in overcoming the G_1/S transition (Floros *et al.* 1981). Thus, it is very probable that every time T-ag reaches the nucleus at the end of mitosis, it might induce cells to enter a new proliferative cycle. As a consequence, cells would be kept continuously proliferating, which is the main feature of transformed cells.

We thank J. S. Butel for the gift of cells and antisera, J. Sans for technical advice, N. Galanti for his invaluable contribution in setting up tissue culture facilities, and R. López for helpful discussion and editing.

This work was supported by Research grants 1137/85, 447/87 from FONDECYT; B2366 from DIB-University of Chile and 59-CHL-24 from The Third World Academy of Sciences.

References

Baserga, R. & Malamud, D. (1969). Light microscopic autoradiography. In *Autoradiography: Techniques and Application* (ed. R. Baserga & D. Malamud). Harper and Row, New York.

Bhorjee, J. S., Barclay, S. L., Wedrychowski, A. & Smith, A. M. (1983). Monoclonal antibodies specific for tight binding human chromatin antigens reveal structural rearrangements within the nucleus during the cell cycle. *J. Cell Biol.* **97**, 389–396.

Bludau, H., Kopun, M. & Werner, D. (1986). Cell cycle-dependent expression of nuclear matrix proteins of Ehrlich ascites cells studied by in vitro translation. *Expl Cell Res.* **165**, 269–282.

Bravo, R., Frank, R., Blundell, P. A. & MacDonald-Bravo, H. (1987). Cyclin/PCNA is the auxiliary protein of DNA polymerase-δ. *Nature, Lond.* **326**, 515–517.

Brockman, W. W. (1978). Transformation of BALB/c-3T3 cells by tsA mutants of simian virus 40: temperature sensitivity of the transformed phenotype and retransformation by wild type virus. *J. Virol.* **25**, 860–870.

Campbell, J. (1988). Eukaryotic DNA replication: yeast bares its ARSs. *Trends Biochem. Sci.* **13**, 212–217.

Carri, M. T., Micheli, G., Graziano, E., Pace, T. & Buongiorno-Nardelli, M. (1986). The relationship between chromosomal origins of replication and the nuclear matrix during the cell cycle. *Expl Cell Res.* **164**, 426–436.

Celis, J. E. & Celis, A. (1985). Cell-cycle dependent variations in the distribution of the nuclear protein cyclin, proliferating cell nuclear antigen, in cultured cells: subdivision of S phase. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3262–3266.

Chaly, N., Bladon, T., Setterfield, G., Little, J. E., Kaplan, J. G. & Brown, D. L. (1984). Changes in distribution of nuclear matrix antigens during the mitotic cell cycle. *J. Cell Biol.* **99**, 661–671.

Chang, H. L. & Baserga, R. (1977). Time of replication of genes responsible for a temperature-sensitive function in a cell cycle-specific ts mutant from a hamster cell line. *J. cell. Physiol.* **92**, 333–344.

Floros, J., Jonak, G., Galanti, N. & Baserga, R. (1981). Induction of cell DNA replication in G1 specific ts mutant by microinjection of SV40 DNA. *Expl Cell Res.* **132**, 215–223.

Galanti, N., Jonak, J. G., Soprano, K. J., Floros, J., Kaczmarek, L., Weissman, S., Reddy, V. B., Tilghman, S. M. & Baserga, R. (1981). Characterization and biological activity of cloned simian virus 40 DNA fragments. *J. biol. Chem.* **256**, 6469–6474.

Gerace, L., Blum, A. & Blobel, G. (1978). Immunocytochemical localization of the major polypeptides of the nuclear pore complex–lamina fraction: interphase and mitotic distribution. *J. Cell Biol.* **79**, 546–566.

Graessman, M. & Graessman, A. (1976). Early simian virus 40-specific RNA contains information for tumor antigen formation and chromatin replication. *Proc. natn. Acad. Sci. U.S.A.* **73**, 366–370.

Harper, F., Florentin, Y. & Puvion, E. (1985). Large T-antigen-rich viral DNA replication loci in SV40-infected monkey kidney cells. *Expl Cell Res.* **161**, 434–444.

Jackson, D. A. & Cook, P. R. (1986). Replication occurs at a nucleoskeleton. *EMBO J.* **5**, 1403–1410.

Kit, S., Kurimura, T. & Dubbs, D. R. (1969). Transplantable mouse tumor line induced by injection of SV40-transformed mouse kidney cells. *Int. J. Cancer* **4**, 384–392.

Kurki, P., Vanderlaan, M., Dolbeare, F., Gray, J. & Tan, E. M. (1986). Expression of proliferating cell nuclear antigen (PCNA)/cyclin during the cell cycle. *Expl Cell Res.* **166**, 209–219.

Lanford, R. E. & Butel, J. S. (1979). Antigenic relationship of SV40 early proteins to purified large T polypeptide. *Virology* **97**, 295–306.

Lanford, R. E. & Butel, J. S. (1981). Effect of nuclear localization of large tumor antigen on growth potential of SV40-transformed cells. *Virology* **110**, 147–158.

Madsen, P. & Celis, J. E. (1985). S-phase patterns of cyclin (PCNA) antigen staining resemble topographical patterns of DNA synthesis. *FEBS Lett.* **193**, 5–11.

Mak, S. (1965). Mammalian cell cycle analysis using microspectrometry combined with autoradiography. *Expl Cell Res.* **39**, 286–289.

Pope, J. H. & Rowe, W. P. (1964). Detection of a specific antigen in SV40-transformed cells by immunofluorescence. *J. exp. Med.* **120**, 121–128.

Prelich, G. & Stillman, B. (1988). Coordinated leading and lagging strand synthesis during SV40 DNA replication in vitro requires PCNA. *Cell* **53**, 117–126.

Prelich, G., Tan, C.-K., Kostura, M., Mathews, M. B., So, A. G., Downey, K. M. & Stillman, B. (1987). Functional identity of proliferating cell nuclear antigen and a DNA polymerase-δ auxiliary protein. *Nature, Lond.* **326**, 517–520.

Rapp, F., Butel, J. S. & Melnick, J. L. (1964). Virus induced intranuclear antigen in cells transformed by papovavirus SV40. *Proc. Soc. exp. Biol. Med.* **116**, 1131–1135.

Rigby, P. W. J. & Lane, D. P. (1983). Structure and function of simian virus 40 large T-antigen. In *Advances in Viral Oncology*, vol. 3 (ed. G. Klein), pp. 31–57. Raven Press, New York.

Ringertz, N., Hadlaczky, G., Hallman, H., Nyman, U., Petterson, I. & Sharp, G. C. (1986). Computer analysis of the distribution of nuclear antigens: studies on the spatial and functional organization of the interphase nucleus. *J. Cell Sci. Suppl. 4*, 11–28.

Santos, M. & Butel, J. S. (1982). Association of SV40 large tumor antigen and cellular proteins on the surface of SV40-transformed mouse cells. *Virology* **120**, 1–17.

Santos, M. & Butel, J. S. (1985). Surface T-antigen expression in simian virus 40-transformed mouse cells: Correlation with cell growth rate. *Molec. cell. Biol.* **5**, 1051–1057.

Soprano, K. J., Galanti, N., Jonak, G. J., McKercher, S., Pipas, J. M., Peden, K. W. C. & Baserga, R. (1983). Mutational analysis of simian virus 40 T-antigen: Stimulation of cellular DNA synthesis and activation of rRNA genes by mutants with deletions in the T-antigen gene. *Molec. cell. Biol.* **3**, 214–219.

Spector, D. L. & Smith, H. C. (1986). Redistribution of U-snRNPs during mitosis. *Expl Cell Res.* **163**, 87–94.

Stahl, H., Drogue, P. & Knippers, R. (1986). DNA helicase activity of SV40 large tumor antigen. *EMBO J.* **5**, 1939–1944.

STAHL, H. & KNIPPERS, R. (1987). The simian virus 40 large tumor antigen. *Biochim. biophys. Acta* **910**, 1–10.

STAUFENBIEL, M. & DEPPERT, W. (1983). Different structural systems of the nucleus are targets for SV40 large T antigen. *Cell* **33**, 173–181.

STERNBERGER, L. A., HARDY, P. H. JR & MEYER, H. G. (1970). The unlabeled antibody-enzyme method of immunocytochemistry. Preparation and properties of soluble antigen antibody complex (horseradish peroxidase–antiperoxidase) and its use in identification of spirochetes. *J. Histochem. Cytochem.* **18**, 315–333.

TJIAN, R., FEY, G. & GRAESSMAN, A. (1978). Biological activity of purified simian virus 40 T-antigen proteins. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1279–1283.

TODOROV, I. T., PHILIPOVA, P. N., ZHELEV, N. Z. & HADJIOLOV, A. A. (1988). Changes in a nuclear matrix antigen during the cell cycle: interphase and mitotic cells. *Biol. Cell* **62**, 105–111.

(Received 5 October 1988 – Accepted, in revised form, 4 April 1989)