Localization of SV40 T antigen in mitotic cells by an immunoperoxidase method

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Summary. The immunoperoxidase technique has clearly demonstrated that SV40 T antigen is dissociated from the chromosomes in mitotic cells, and massive transport of T antigen from the cytoplasm to the nucleus appears to take place during or immediately after the telophase.

The tumor (T) antigen of simian virus 40 (SV40) has been extensively studied since Black et al.[1] first detected T antigen in SV40-transformed cells. The T antigen is generally believed to be associated with the nuclear chromatin of SV40-transformed cells[2]. Recent investigations have clarified that the SV40 T antigen is the product of the A gene of the SV40 genome and is necessary for the initiation of viral DNA replication, the regulation of viral gene expression and the establishment and also maintenance of virus-induced neoplastic transformation[3-5]. 2 forms of SV40 T antigen have been characterized to date. They are called large T and small t antigens, and have mol. wts in the ranges 90,000-100,000 and 15,000-20,000, respectively[6]. It is also known that the amount of T antigen in SV40-transformed cells increases during DNA replication and is approximately twice as large in G2 as in G1 nuclei[7]. There have been several immunocytochemical studies[8-11] on the localization of T antigen in SV40-transformed cells in interphase since Pope et al.[12] first observed T antigen in the nuclei but not nucleoli of SV40-transformed cells by an immunofluorescence method. However, there is no report regarding either the rearrangement or the ultrastructural location of T antigen in SV40-transformed cells during mitosis. The present study is concerned with the immunoperoxidase localization of SV40 T antigen detached from the chromosomes in mitotic cells.

Materials and methods. Clonal cells (C5A)[13] originally derived from SV40 (wild type, strain RH-911)-transformed brain cells of hamster embryo were used. They were maintained in Ham's F-12 medium supplemented with 5% fetal calf serum and antibiotics. The C5A cells in various cell cycle phases grown on chamber slides were processed for immunocytochemistry at both light and electron microscopic levels, using horseradish peroxidase-labelled Fab' fragments as detailed previously[14]. Thus stained, the C5A cells in mitosis were selected under the light microscope, then cut on an ultramicrotome and photographed in a Hitachi HS-8 electron microscope without counterstain.

Results and discussion. By light microscopy, the positive immunoperoxidase staining for T antigen as a dark accumulation of diaminobenzidine reaction precipitates is localized exclusively in the nuclei (except for the nucleoli) of

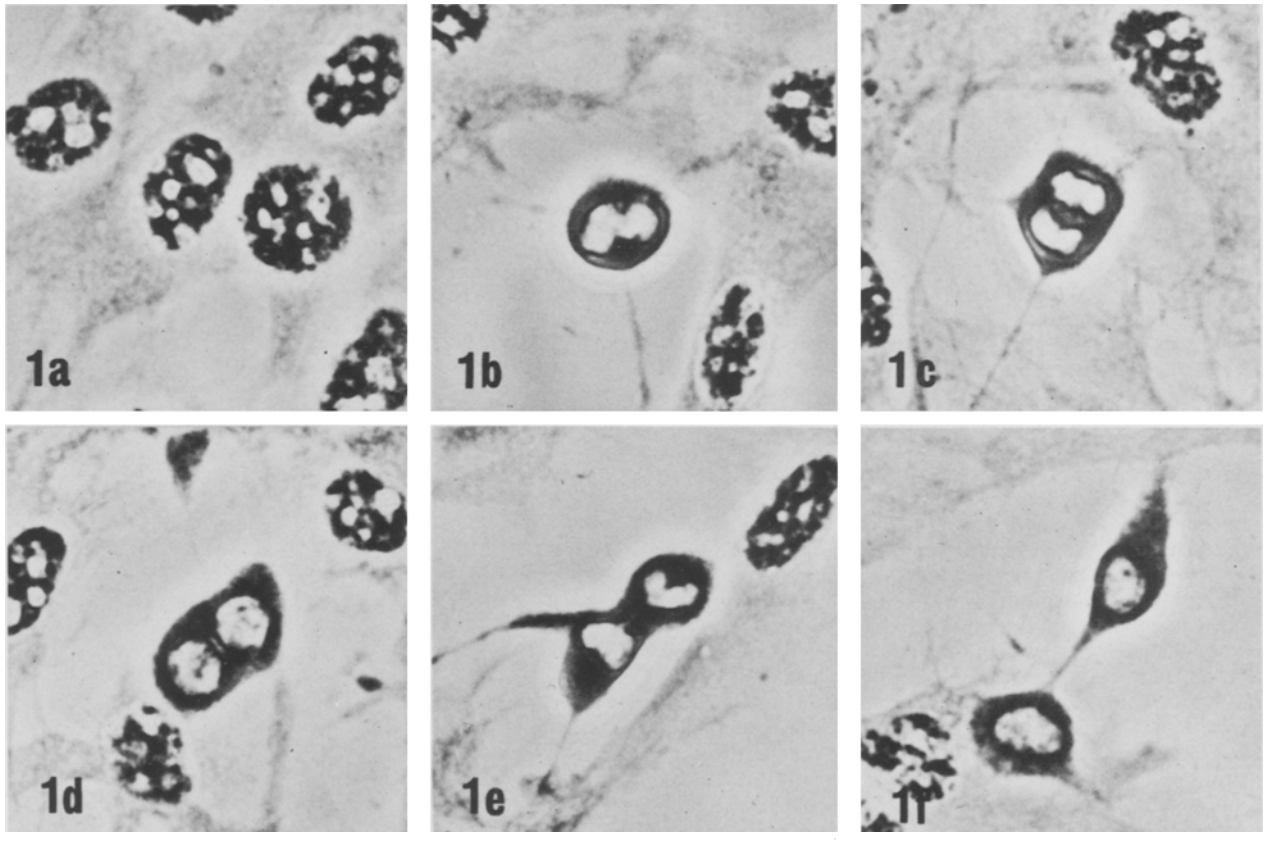

Fig. 1. By light microscopy, the positive immunoperoxidase staining for SV40 T antigen is seen as a dark accumulation of diaminobenzidine reaction products in the nuclei (except for nucleoli) of C5A cells in interphase (a), while the positive staining for T antigen is virtually distributed throughout the cytoplasms with no staining in the chromosomal areas of mitotic cells (b, c, d, e, f). × 400.

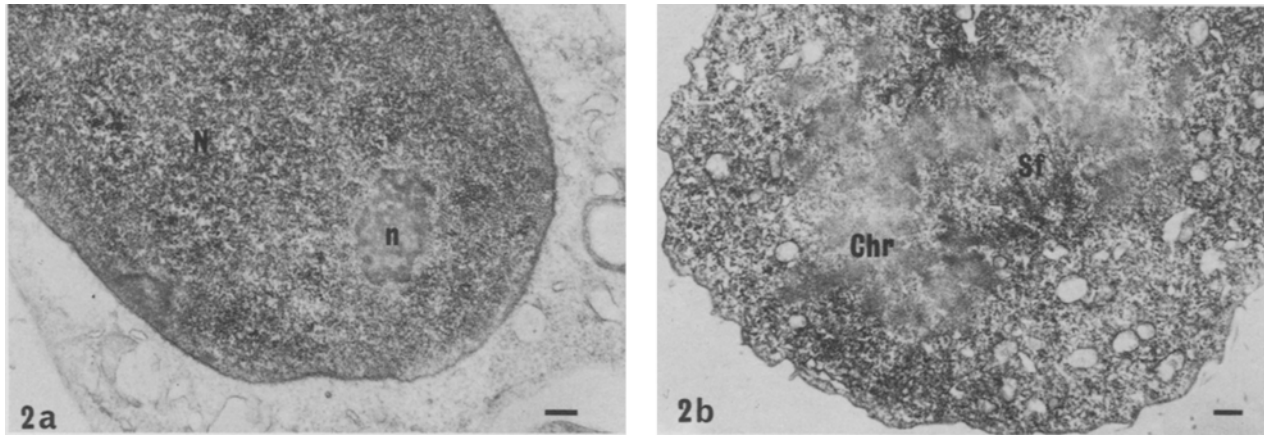

Fig. 2. By electron microscopy, the electron-dense reaction precipitates as the positive staining for T antigen are seen exclusively in the nucleus (apart from the nucleolus) of C5A cell in interphase (a). On the other hand, T antigen is distributed throughout the cytoplasm of mitotic cells and the condensed chromosomes do not seem to be tagged with the reaction products (b). Bar = 1 μm, N = nucleus, n = nucleolus, Chr = chromosomes, Sf = spindle fibre.

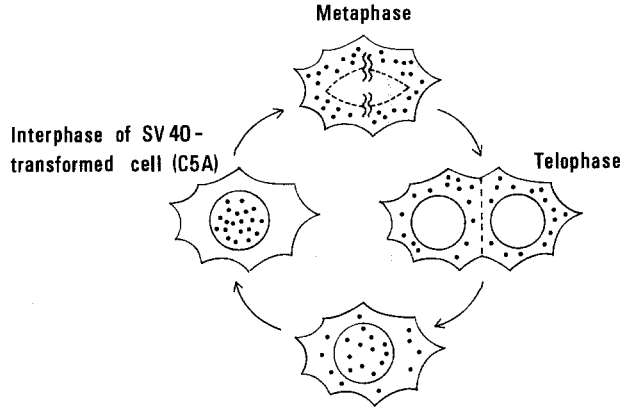

Fig. 3. The massive transport of T antigen (represented as small dots) from the cytoplasm to the nucleus appears to take place during or immediately after telophase in the mitosis of the C5A cell.

C5A cells in interphase (figure 1,a); however, in mitotic cells, the positive staining for T antigen is virtually distributed throughout the cytoplasm with no staining in the chromosomal area (figure 1,b–f). By electron microscopy, the electron-dense reaction products of the positive immunoperoxidase staining for T antigen are seen solely in the nucleus (except for the nucleolus) in C5A cells in interphase (figure 2,a). On the other hand, in the mitotic cell, T antigen is distributed throughout the cytoplasm, while the condensed chromosomes do not seem to be tagged with the immunoperoxidase reaction products for T antigen (figure 2,b), confirming the results obtained at the light microscopic level. It is of special interest that the spindle fibres appear to be highly associated with T antigen. The Fab' fragments used in this study are assumed to be against both large T and small t antigens, since the C5A cells are derived from hamster brain cells transformed with the wild type SV40 and the antisera were obtained from hamsters bearing the C5A tumor. Therefore, the authors interpret that the present immunoperoxidase results show simultaneously the location of both large T and small t antigens in C5A cells. At the present time, the enzyme-labelled antibody method is thought to be the most suitable technique for analyzing the ultrastructural location of T antigen. We showed in a previous report[11] that T antigen in both SV40-transformed and infected cells could be precisely and clearly demonstrated using enzyme-labelled antigen-binding Fab' fragments, which penetrated more easily into the fixed cells. By application of this sensitive immunoperoxidase technique, it seems that SV40 T antigen is dissociated from the chromosomes in mitotic cells and massive transport of T antigen from the cytoplasm to the nucleus takes place during or immediately after the telophase in C5A cells as shown schematically in figure 3. The ultrastructural location of T antigen as a loose network in the nucleoplasm (apart from the nucleoli) in the interphase cell is considered to represent an association of the antigen with nuclear chromatin[5,11], supporting the evidence that SV40 T antigen preferentially binds not only to SV40 DNA at or near the origin of DNA replication[15] but also to double stranded DNA[16]. Although the functional significance of T antigen being detached from the chromosomes during mitosis remains to be resolved, it may relate to the mechanism of self-regulated synthesis of T antigen.

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