JOURNAL OF VIROLOGY, Sept. 1989, p. 3651–3660
0022-538X/89/093651-10$02.00/0

Vol. 63, No. 9

Interactions of Minute Virus of Mice and Adenovirus with Host Nucleoli

TRACY H. WALTON, PHILLIP T. MOEN, JR., ELIZABETH FOX, AND JOHN W. BODNAR*

Department of Biology, Northeastern University, Boston, Massachusetts 02115

Received 13 January 1989/Accepted 5 May 1989

Biochemical evidence is presented that both minute virus of mice (MVM) and adenovirus interact with the nucleolus during lytic growth and that MVM can also target specific changes involving nucleolar components in adenovirus-infected cells. These virus-nucleolus interactions were studied by analysis of intranuclear compartmentalization of both viral DNAs and host nucleolar proteins: (i) MVM in mouse cells (its normal host) replicates its DNA in the host nucleoli; (ii) specific nucleolar proteins as well as small nuclear ribonucleoprotein antigens are recompartmentalized to multiple intranuclear foci in adenovirus-infected HeLa cells; and (iii) when adenovirus helps MVM DNA replication in a nonpermissive human cell (HeLa), the MVM DNA is also recompartmentalized for synthesis. The data suggest mechanisms for disruption of nucleolar function common to oncogenic or oncolytic virus lytic growth and cell transformation.

The nucleolus is an intranuclear organelle which is the site of synthesis for rRNAs and of assembly of ribosome subunits (25, 49). Several hundred copies of the genes for rRNAs are tandemly repeated in the DNA contained in the mammalian nucleolus (31, 48). Electron microscopy studies with parvoviruses H-1 and minute virus of mice (MVM) have indicated involvement of nucleoli in parvovirus lytic growth (1, 40, 43–45). Electron microscopy studies with adenovirus have shown that adenovirus DNA is replicated in multiple foci throughout the nucleus called "replication factories" which are not nucleoli (33, 55). However, host nucleoli are degraded (38) and rRNA synthesis is inhibited during adenovirus infection (5, 29). These data suggest interactions of both viruses with host nucleoli. This hypothesis was tested by characterizing the intranuclear localization of MVM and adenovirus DNAs and of host nucleolar proteins.

We found that both MVM and adenovirus type 2 (Ad2) interact with host nucleoli, but in different ways. MVM directly targets the nucleoli in that MVM in its normal mouse host cells replicates its DNA in the nucleoli. Adenovirus interacts with the nucleoli by recompartmentalizing nucleolar components to the replication factories to form "pseudonucleoli." Also, small nuclear ribonucleoprotein (snRNP)-associated antigens are recompartmentalized to these intranuclear foci. Adenovirus can help MVM DNA replication in human HeLa cells, and when this occurs, the MVM DNA is also recompartmentalized by Ad2. Our data suggest that intranuclear compartmentalization of DNA may be important for replication of both MVM and Ad2 and that there are mechanisms involving nucleoli which are shared by these oncolytic and oncogenic viruses. These pathways for disruption of nucleolar function may also be shared in mechanisms of oncogenesis.

MATERIALS AND METHODS

Cells and viruses. Mouse A9 cells were provided by David Ward (Yale University). HeLa cells were provided by Marie Chow (Massachusetts Institute of Technology). The prototype strain of MVM (MVM[p]) was provided by David Ward, and Ad2 was provided by George Pearson (Oregon State University).

Cell lines were grown in Autopow minimum essential medium (Flow Laboratories, Inc.) supplemented with 10% fetal calf serum and glucose (4.9 g/liter). Viral infections (9, 52) were performed on cells grown directly on microscope slides (Cell-Tech Inc.). Since MVM replicates only during S phase, cells need to be synchronized to ensure that the maximal number of cells are synchronously infected with MVM; cells were treated similarly during adenovirus infections to provide consistency with the MVM-plus-Ad2 infections. Experiments with unsynchronized Ad2-infected HeLa cells gave similar results. Cells were parasynchronized with 0.2 mM thymidine for 12 to 18 h (9, 53), the thymidine-containing medium was removed, viruses (MVM or Ad2 or both) were added at a multiplicity of infection of 10 to 100 PFU per cell, and the cells were then grown for 24 h.

Localization of proteins and DNA in situ. Antisera against MVM NS-1 (10) and MVM capsids were provided by Susan Cotmore and Peter Tattersall (Yale University). Antiserum to MVM terminal protein (TP) was raised by using purified MVM DNA-TP complexes (9). Antiserum to nucleolin was provided by Mark Olson (University of Mississippi Medical Center). Antiserum to protein B23 was provided by P. K. Chan and Harris Busch (Baylor College of Medicine). Antiserum to Ad2 72-kilodalton (kD) DNA binding protein (DBP) was provided by Vaughn Cleghon and Daniel Klessig (Waksman Institute, State University of New Jersey). Antisera to RNA polymerase I, NOR90, Th, U3, Sm, U2, and Ku were provided by Susan Baserga and Joan Steitz (Yale University). Antisera AF/CDC3 (U1), -6 (nucleolar), -8 (centromere), and -9 (topoisomerase I) were obtained from the Centers for Disease Control. Fluorescein isothiocyanate (FITC)-conjugated antibodies to Ad2 virions were obtained from Chemicon International. Cloned MVM DNA (pMM984) as described by Merchlinsky et al. (32) was provided by David Ward.

Proteins were detected in situ in cells grown on microscope slides, parasynchronized with 0.2 mM thymidine as

* Corresponding author.

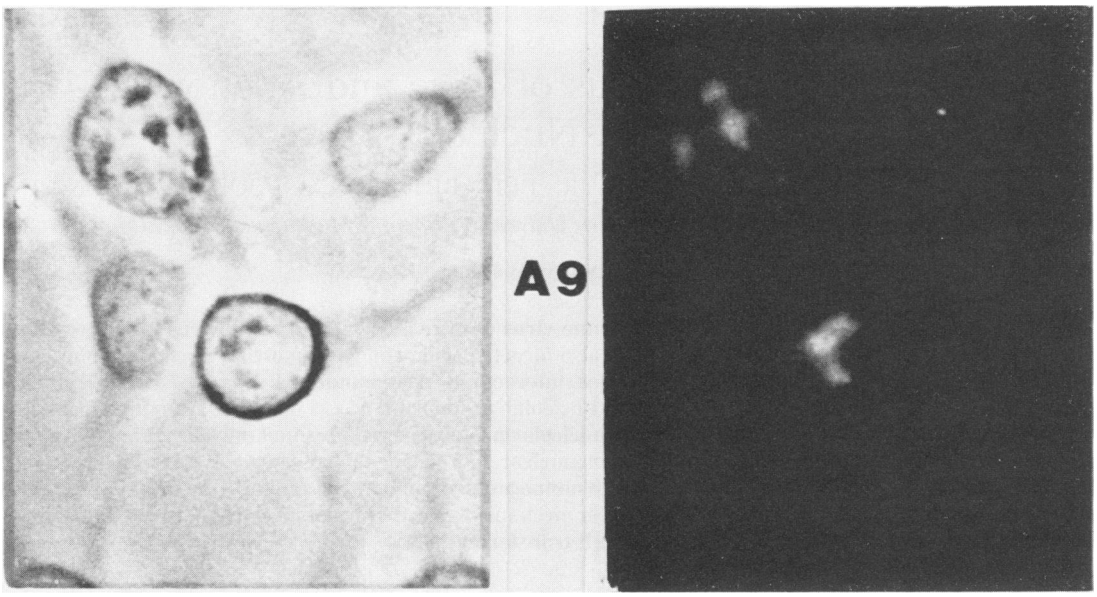


FIG. 1. Intranuclear localization of MVM DNA in mouse fibroblasts. Mouse (A9) cells were grown on slides (Cell-Tech Inc.), parasynchronized with a single thymidine block, and infected with MVM at a multiplicity of infection of 20 upon release from the block. The infected cells were fixed at 18 h postinfection and probed in situ with biotinylated MVM DNA, and the biotinylated DNA was developed with FITC-conjugated streptavidin. The cells were then photographed under phase-contrast optics (left) or UV light (right).

above (9, 53), infected with MVM, Ad2 (37, 52), or MVM plus Ad2 (10 to 100 PFU/cell), and grown for 24 h. The cells were washed in phosphate-buffered saline (PBS) and fixed in methanol-acetone (1:2). The fixed cells were washed five times for 2 min each in PBS and then incubated for 1 h at 37°C in the appropriate antibody or mixture of antibodies diluted in PBS. The cells were rinsed five times for 2 min each at room temperature and then incubated for 30 min at 37°C with the appropriate second antiserum or mixture of fluorescently tagged antisera at a dilution of 1:50 in PBS. The conjugated second antisera for double-labeling experiments were as follows: anti-DBP used tetramethyl-rhodamine-isothiocyanate (TRITC)–anti-rabbit immunoglobulin; anti-B23 and anti-Sm used FITC–anti-mouse immunoglobulin; anti-NOR90, -polymerase I, -CDC6, -Th, -U3, -U1, -U2, -Ku, and -topoisomerase I and anticentromere used TRITC–anti-human immunoglobulin. In each case, anti-TP was developed with TRITC– or FITC–anti-guinea pig immunoglobulin of the alternate conjugate to the first antiserum. The slides were then washed in PBS at room temperature and fixed for photography in PBS-buffered glycerol.

DNA was detected in situ in cells grown on microscope slides (4, 21, 46). Cells were infected as described above. The cells were washed in PBS and fixed in 4% paraformaldehyde in PBS for 30 min. The cells were then washed three times in PBS and dehydrated through 30, 60, 80, and 95% and absolute ethanol, 5 min each, and then air dried. The fixed dehydrated cells were stored at 4°C until probed.

Immediately prior to probing, the cells were rehydrated in PBS, followed by a 10-min acid treatment in 0.02 N HCl. The cells were washed twice in PBS, and the cell membranes were detergent solubilized in 0.01% Triton X-100 in PBS for 90 s. Following two PBS washes, the cells were treated in DNase-free pronase (0.1 mg/ml in 50 mM Tris hydrochloride [pH 7.5], 5 mM EDTA; Calbiochem-Behring) at room temperature for 8 min. The pronase treatment was halted by immersing the cells in PBS-glycine (2 mg/ml) three times, 5 min each. A second 4% paraformaldehyde fixation was performed for 5 min, followed by three 5-min washes in PBS-glycine. The cells were dehydrated through 30, 60, 80, and 95% and absolute ethanol and air dried.

The DNA used to probe for MVM-viral DNA is a plasmid pTZ18U subclone containing the 937-base *Pst*I C fragment (MVM nucleotides 411 to 1354) derived from plasmid pMM984 (32). This subclone is designated pPM1816. Plasmid pPM1816 was nick translated, using biotin-11–dUTP (Bethesda Research Laboratories, Inc.) as a substrate in place of dTTP. The unincorporated biotin-11–dUTP was removed from the reaction mixture by Sephadex G-50 chromatography, and the biotin-labeled DNA was vacuum dried. The in situ hybridization mixture consists of 50% (vol/vol) deionized formamide, 10% (wt/vol) dextran sulfate, 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), pH 8.0, 25 mg of sheared herring sperm DNA per ml as carrier, and 2 μg of biotin-labeled pPM1816 DNA per ml.

The in situ hybridization was performed by denaturing the MVM or Ad2 target DNA in situ by placing the fixed and dried slides in 95% deionized formamide at 65°C for 10 min, followed by placement on an ice-cold aluminum plate to cool the slides rapidly. This step helps to prevent self-reannealing of the target DNA. The biotin-labeled pPM1816 DNA was denatured at 95°C for 5 min, followed by rapid cooling on ice for 10 min. A 20-μl portion of the hybridization mixture was pipetted onto each well, and a cover slip was gently placed on top of the well. Hybridization was at 37°C for 12 to 14 h. Following hybridization, the cover slips were gently floated off the slides in 2× SSC at room temperature. The cells were then washed twice in 50% deionized formamide–2× SSC at 34°C for 5 min each, followed by two 5-min washes in 2× SSC at 34°C and then two 5-min washes in 2× SSC at room temperature. The biotin-labeled DNA was detected by incubating the cells in either fluorescein- or Texas red-conjugated streptavidin (Bethesda Research Laboratories) in 1.5% bovine serum albumin in PBS for 1 h at 37°C, followed by two 5-min PBS washes.

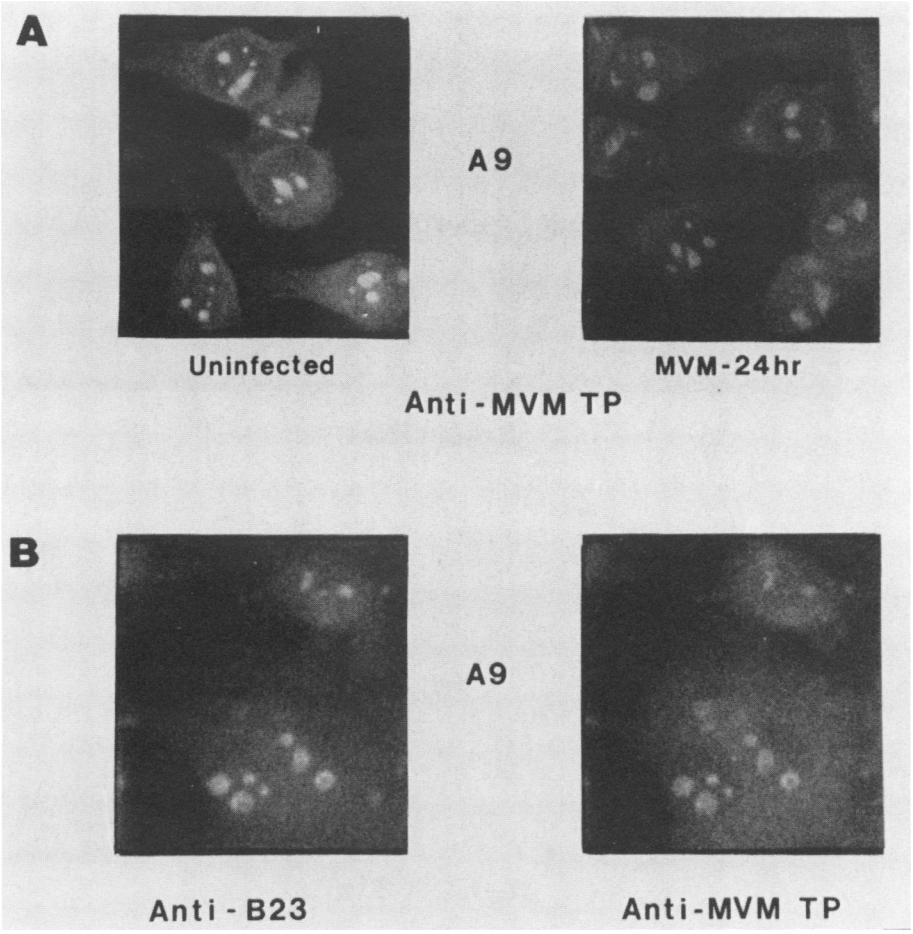


FIG. 2. MVM interactions with mouse nucleoli. (A) Localization of MVM TP in uninfected (left) or MVM-infected (right) mouse A9 cells. Uninfected or MVM-infected cells were harvested 24 h postinfection and fixed with methanol-acetone (1:2). The fixed cells were probed with antiserum to the MVM TP raised in a guinea pig (1:100 dilution) and developed with FITC-conjugated anti-guinea pig immunoglobulin serum (1:50; Kirkegard and Perry). (B) Colocalization of nucleolar antigen B23 and MVM TP. Uninfected A9 cells were fixed as before and probed with anti-MVM TP serum and with mouse antiserum to nucleolar antigen B23 (1:100). The immune complexes were visualized with a mixture of rhodamine-conjugated anti-mouse (left) and FITC-conjugated anti-guinea pig (right) immunoglobulin serum.

RESULTS

The role of host nucleoli in the lytic infections of MVM and Ad2 was investigated by study in situ of the intranuclear compartmentalization of both MVM and Ad2 DNAs and host nucleolar proteins.

MVM in mouse cells. MVM DNA is compartmentalized spatially within the nuclei of mouse (A9) fibroblasts. MVM DNA was detected in situ at 18 h postinfection (Fig. 1), using a biotinylated MVM DNA probe; the biotinylated DNA was developed with FITC-conjugated streptavidin (4, 21, 46). Comparison of the phase-contrast and fluorescence pictures indicated that the MVM DNA is localized within the host nucleoli. As infection proceeds, the nucleoli degrade and the MVM DNA in situ begins to fill up the nucleus (data not shown). MVM DNA is associated with the host nuclear matrix through the MVM TP; the nuclear matrix attachment sites become saturated at 16 to 20 h postinfection, and after that time the MVM replicative form DNA accumulates in the nucleoplasm (J. W. Bodnar and D. Ward, submitted for publication). The nucleolar degradation and filling up of the nucleus with MVM DNA begin about the same time as the saturation of nuclear matrix attachment sites, suggesting that the nucleoli are the functional sites of DNA replication and that viral infection saturates the nucleolar machinery on the nuclear matrix at 16 to 20 h postinfection.

We also investigated the intranuclear compartmentalization of MVM TP. The MVM replicative form DNAs have a 60-kilodalton (kDa) protein, TP, covalently bound to their 5′ termini. Antiserum was raised against MVM replicative form DNA-TP complexes (2, 9). This antiserum, which can immobilize MVM DNA-TP complexes on protein A-Sepharose columns (9), reacts with both uninfected and MVM-infected mouse cells, indicating that the protein bound to MVM DNA is a host polypeptide (see below). Cotmore and Tattersall (11) have shown that antisera raised to the MVM-encoded nonstructural protein NS-1 also react to proteins bound to the MVM DNA termini. Therefore, current data indicate that there is a complex of MVM NS-1 and a 60-kDa host protein bound to the MVM DNA termini. For consistency with the literature (2, 9), we refer to the 60-kDa host protein as TP, realizing that it is specifically bound to the MVM DNA termini either directly or indirectly through NS-1.

In situ detection of the MVM TP (Fig. 2A) indicated that the TP is found in discrete foci in the nucleus of both

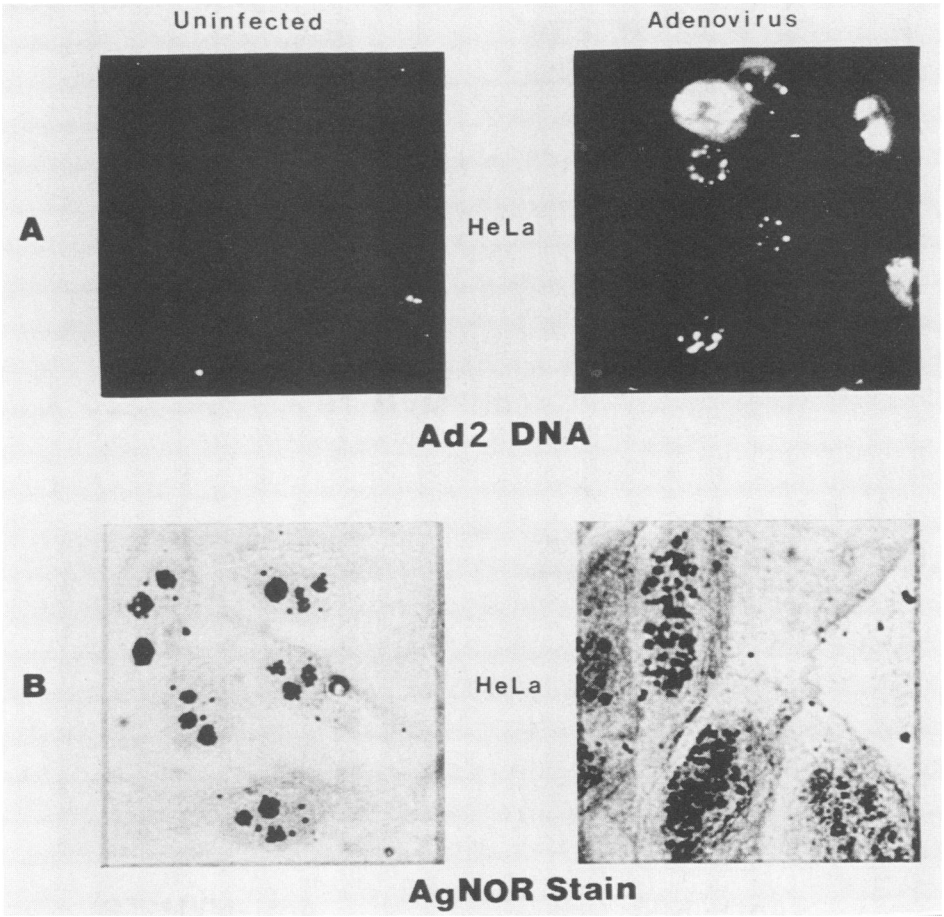


FIG. 3. Recompartmentalization of nucleolar components in adenovirus-infected HeLa cells. (A) Localization of Ad2 DNA to replication factories. HeLa cells were grown on slides and infected with Ad2 (multiplicity of infection, 10). The cells were fixed at 24 h postinfection, probed with biotinylated Ad2 DNA, and developed with FITC-conjugated streptavidin. (B) Recompartmentalization of silver-staining nucleolar components in Ad2-infected HeLa cells. HeLa cells were infected on slides as before and fixed in methanol. Treatment with silver nitrate (24) was used to stain the AgNOR-staining components of the nucleoli in uninfected (left) or Ad2-infected (right) cells.

uninfected and MVM-infected cells. Since the intensity of the signal is unchanged in MVM infection, TP does not appear to be induced by MVM infection. This was confirmed by Western blot (immunoblot) analysis of the MVM TP throughout infection (data not shown). The foci containing the MVM TP are nucleoli as confirmed by double-labeling experiments with the control nucleolar markers B23 (numatrin; 14, 15) and nucleolin (C23; 23, 25, 36, 39). MVM TP and B23 (Fig. 2B) as well as nucleolin (data not shown) colocalized within mouse A9 cells, indicating that the TP is in fact a nucleolar antigen. Like B23 (35), the MVM TP was dispersed throughout the cell during mitosis.

Adenovirus in HeLa Cells. Adenovirus also interacts with the host nucleoli during its lytic infection, but in a different way. Adenovirus DNA is replicated in multiple foci throughout the nucleus (38, 55) as detected by biotinylated Ad2 probes (Fig. 3A) and FITC-conjugated streptavidin (4, 21, 46). These replication factories are not nucleoli, as indicated by comparison with phase-contrast pictures (data not shown).

Adenovirus recompartmentalizes nucleolar components to multiple foci throughout the nucleus which have a distribution and appearance similar to those of the replication factories. Nucleoli can be stained with silver nitrate since protein components of the nucleolar organizing regions (NORs) specifically bind silver (24). This AgNOR staining technique was used on both uninfected and Ad2-infected HeLa cells (Fig. 3B). In the uninfected cells, three to six spots were evident corresponding to the nucleoli of the polyploid HeLa cells. In Ad2-infected HeLa cells, the AgNOR stain was in multiple foci characteristic of the adenovirus replication factories.

Since these data pointed to recompartmentalization of nucleolar components by Ad2, we were interested to see how common this phenomenon was and which components colocalized within the nucleus. We studied this by using the control nucleolar marker B23 and a group of antinuclear antibodies (ANAs) from autoimmune sera which reacted with specific nuclear components. We also used antiserum to MVM TP and antiserum to Ad5-encoded 72-kDa DBP (56).

The antiserum to MVM TP detected that antigen extremely weakly in the nucleoli of uninfected HeLa cells (Fig. 4), but in Ad2-infected HeLa cells TP stained much more intensely and was recompartmentalized to multiple foci similar in appearance and distribution to the Ad2 replication factories. To confirm that these foci were replication factories, the distribution of MVM TP was compared with the pattern stained by antiserum to the Ad5 72-kDa DBP. Voelkerding and Klessig have shown that the Ad5 DBP is compartmentalized to multiple intranuclear foci and these

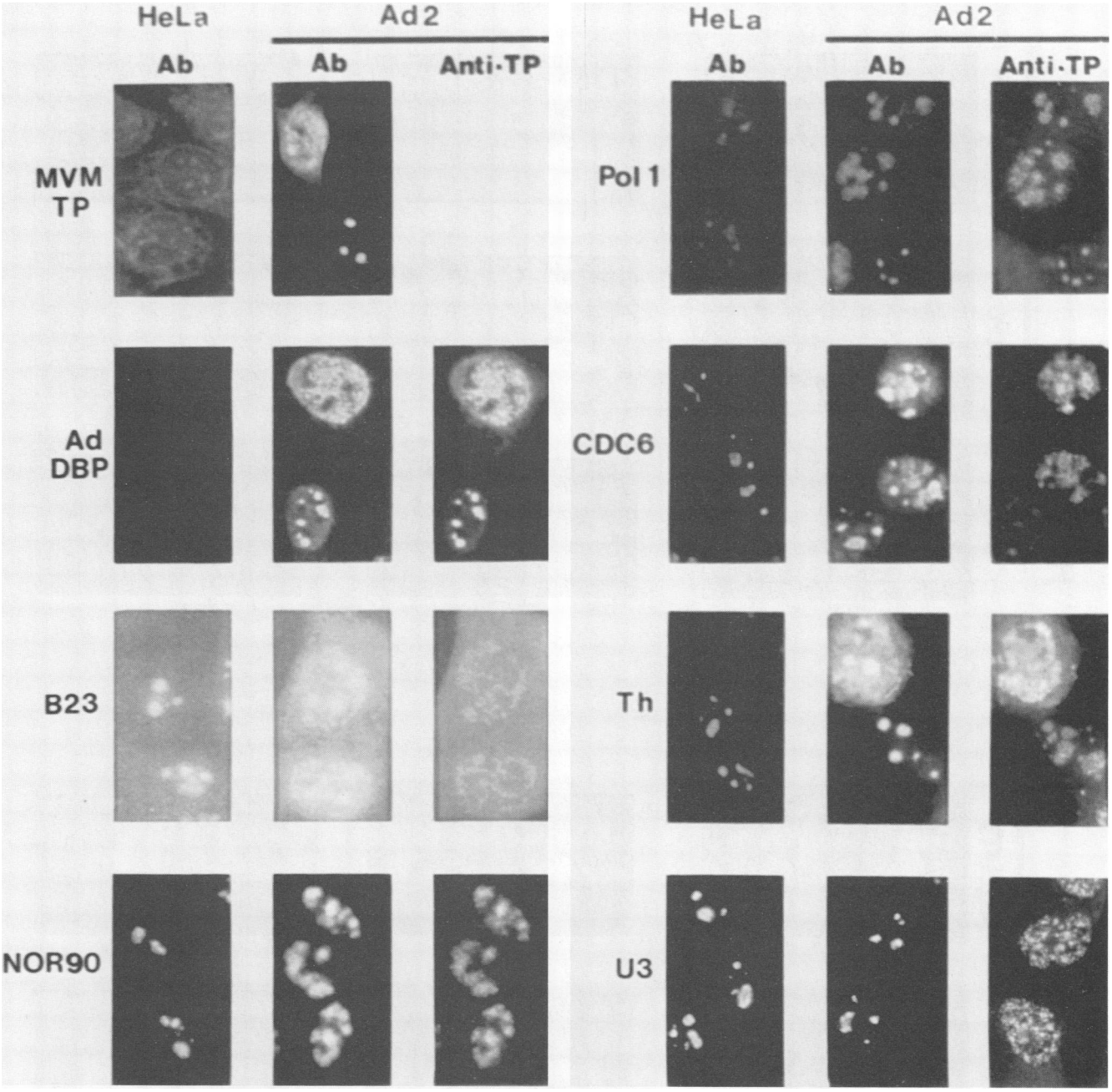
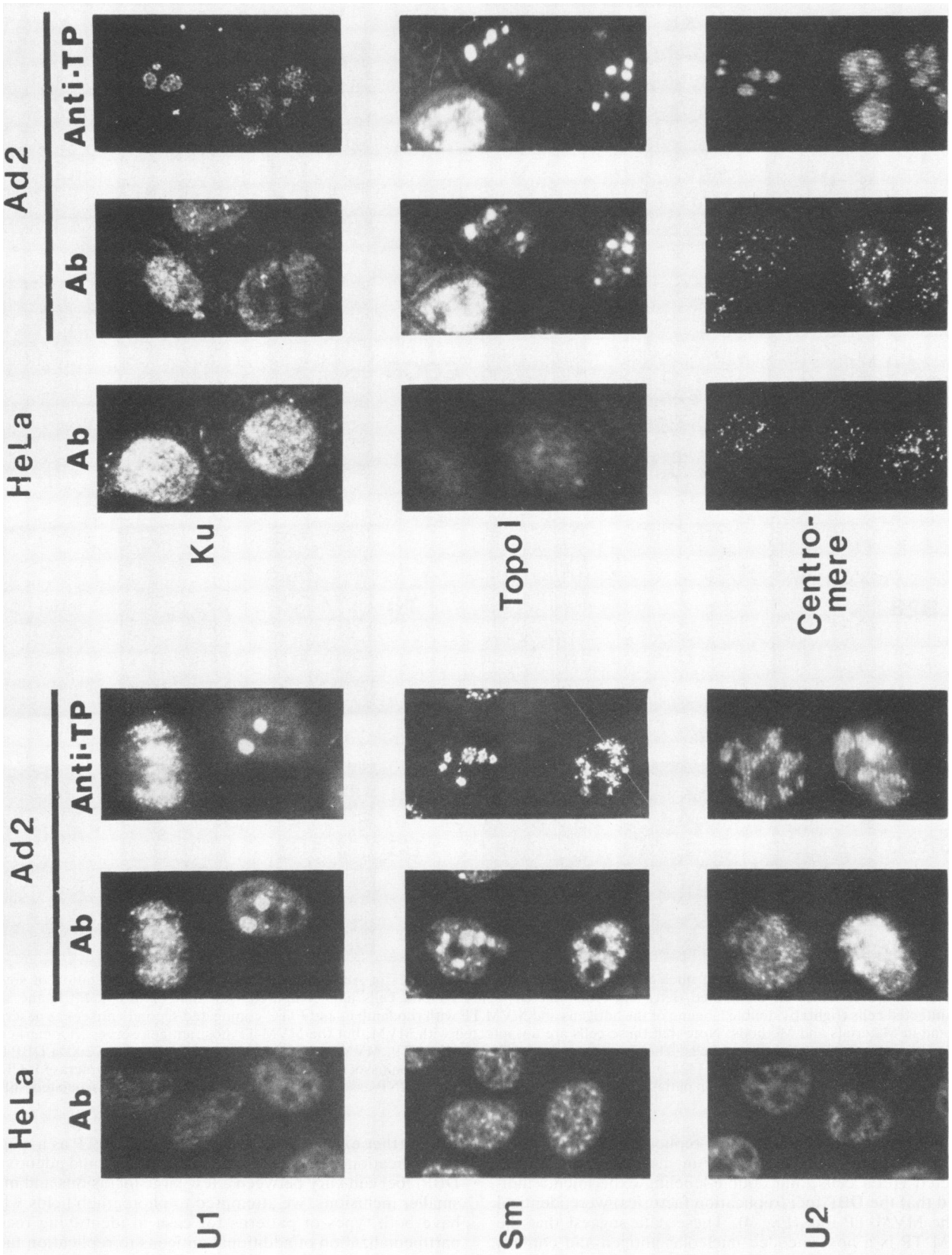


FIG. 4. Recompartmentalization of nucleolar antigens to replication factories in Ad2-infected HeLa cells. The localization of several antigens was determined in uninfected (left) and Ad2-infected (center) HeLa cells and compared with that of MVM TP in the same Ad2-infected cells (right) by double labeling of the antigens and MVM TP with rhodamine- and FITC-conjugated second antisera as described in detail in Materials and Methods. Note that these cells are not infected with MVM, but the MVM TP host antigen serves as a marker for replication factories in Ad2-infected cells. The antisera were as follows: MVM TP, MVM terminal protein; Ad DBP, Ad5 72-kDa DBP (56); B23, nucleolar antigen B23 (numatrin; 14, 15); NOR90, nucleolar organizing region-associated antigen (41); Pol 1, RNA polymerase I; CDC6, control ANA which exhibits nucleolar pattern from CDC (AF/CDC6); Th, snRNP-associated antigen Th (22); U3, U3-associated snRNP antigen (30).

foci colocalize with Ad5 DNA in replication factories (56). The adenovirus DBP was found in multiple foci in Ad2-infected HeLa cells, and double-labeling experiments indicated that the DBP foci (replication factories) were identical to the MVM TP foci (Fig. 4). These data suggest that the MVM TP is a host-encoded nucleolar antigen and confirm that recompartmentalization of that nucleolar protein is to Ad2 replication factories.

In further experiments, we used the MVM TP as a marker for replication factories. Since the MVM TP (and adenovirus DBP) foci can vary between a few large inclusions and many smaller inclusions, we attempted to photograph fields which have both types of patterns for ease in identifying recompartmentalization of additional antigens to replication factories.

We studied the recompartmentalization of nucleolar pro-


HeLa
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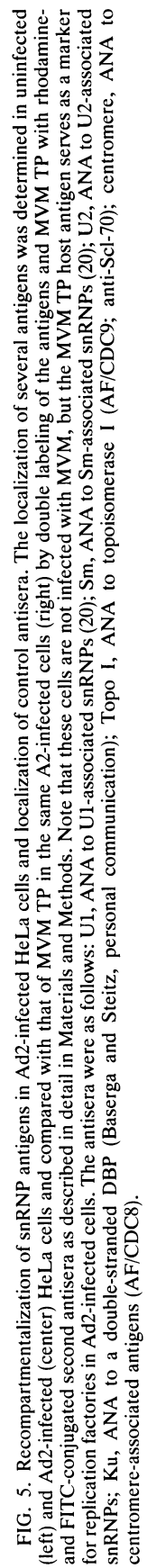

FIG. 5. Recompartmentalization of snRNP antigens in Ad2-infected HeLa cells and localization of control antisera. The localization of several antigens was determined in uninfected (left) and Ad2-infected (center) HeLa cells and compared with that of MVM TP in the same A2-infected cells (right) by double labeling of the antigens and MVM TP with rhodamine- and FITC-conjugated second antisera as described in detail in Materials and Methods. Note that these cells are not infected with MVM, but the MVM TP host antigen serves as a marker for replication factories in Ad2-infected cells. The antisera were as follows: U1, ANA to U1-associated snRNPs (20); Sm, ANA to Sm-associated snRNPs (20); U2, ANA to U2-associated snRNPs; Ku, ANA to a double-stranded DBP (Baserga and Steitz, personal communication); Topo I, ANA to topoisomerase I (AF/CDC9; anti-Scl-70); centromere, ANA to centromere-associated antigens (AF/CDC8).

teins by double-labeling experiments, using a series of antisera and ANAs along with antiserum to MVM TP. To ensure that there was no bleed-through of the red or green fluorescence or cross-reactivity of second fluorescent antisera, all experiments were repeated with a single antiserum, and the results were the same (data not shown).

In general, we found three types of patterns. (i) Nucleolar proteins B23, NOR90, RNA polymerase I, and antigen AF/CDC6 recompartmentalize to replication factories in Ad2-infected HeLa cells. Antiserum to B23 (numatrin) was described above; the ANA to NOR90 recognizes a 90-kDa component of the NOR (41); anti-polymerase I recognizes nucleolar RNA polymerase I; and AF/CDC6 recognizes an uncharacterized nucleolar antigen. All of these antisera detect antigens in the nucleoli of uninfected HeLa cells (Fig. 4). In Ad2-infected HeLa cells, all of these antigens were recompartmentalized to multiple foci, although nucleoli still labeled as before; the foci were determined to be replication factories since they were identical to MVM TP foci in the same cells (Fig. 4). Since the nucleolar fluorescence of these antigens was unchanged in the Ad2-infected cells and the replication factory pattern was at a higher intensity, we suggest that foci may represent de novo synthesis of the antigens during Ad2 infection which is directed to replication factories. However, we cannot discount that this phenomenon may be due to unmasking of antigenic sites during nucleolar degradation and relocalization of the antigens to replication factories.

(ii) Nucleolar snRNP antigen Th is relocalized to a diffuse pattern throughout the nucleus in Ad2-infected HeLa cells. The Th ANA recognizes nucleolar RNPs containing the 7-2, 8-2, and Ro small nuclear RNAs (22). In uninfected HeLa cells, the Th antigen was localized in nucleoli (Fig. 4). In Ad2-infected HeLa cells, Th was still detected in nucleoli but was also seen throughout the nucleus in a diffuse fluorescence distinct from the MVM TP pattern.

(iii) Localization of nucleolar snRNP antigen U3 is unaffected by Ad2 infection. The U3 ANA precipitates RNPs containing the U3 small nuclear RNA (30). The U3 antigen was localized only in nucleoli of both uninfected and Ad2-infected HeLa cells, including those infected cells in which MVM TP was seen in replication factories.

During control experiments for Ad2-induced recompartmentalization, we found the unexpected result that the non-nucleolar snRNP-associated U1 antigen was also recompartmentalized in Ad2-infected HeLa cells. We therefore examined additional ANAs to splicing RNPs. The U1 (AF/CDC3), Sm, and U2 ANAs recognize antigens contained in RNA splicing snRNPs (20). All three of these antigens were found in a speckled non-nucleolar pattern in uninfected HeLa cells (Fig. 5). In Ad2-infected HeLa cells, this pattern remained, but the replication factory pattern (identical to MVM TP) was superimposed on it for U1 and Sm (Fig. 5). For U2, an additional, more intense speckled pattern was superimposed on the original pattern, but the new foci were not the same as the TP foci (Fig. 5). The distribution of the additional Fi ANA to L5/5S snRNPs (51) was unaffected by Ad2 infection (data not shown).

Additional negative and positive control experiments were performed to show that antigen localization to replication factories did not represent nonspecific protein aggregation induced by Ad2 infection. The Ku ANA detects a double-stranded DBP (S. Baserga and J. Steitz, personal communication). This antigen was found in a diffuse nuclear pattern in both uninfected and Ad2-infected HeLa cells (Fig. 5) even when MVM TP was in replication factories, indicating (along

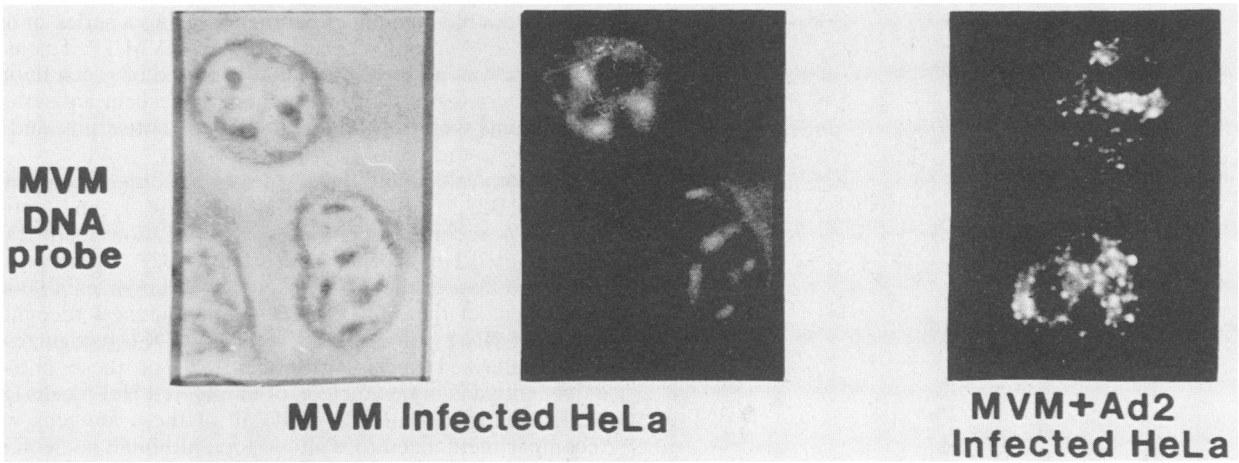

FIG. 6. Localization of MVM DNA in HeLa cells and Ad2-infected HeLa cells. HeLa cells were infected with MVM (multiplicity of infection, 10) or coinfected with MVM and Ad2 (multiplicity of infection, 10 each) as before. The cells were fixed at 24 h postinfection, probed with biotinylated MVM DNA, and detected with Texas red-conjugated streptavidin. MVM-infected HeLa cells were photographed under phase-contrast optics (left) or UV light (center) to indicate nucleolar localization of MVM DNA. Cells coinfected with MVM and Ad2 were probed with MVM DNA and photographed under UV light (right).

with Th and U3 above) that Ad2-induced relocalization to nuclear foci is specific for certain nucleolar and snRNP antigens. Topoisomerase I has been shown to be enriched in nucleoli (34), to interact with transcriptionally active rDNA (12, 25, 34), to nick specifically within Ad2 DNA inverted terminal repeats (7), and to be induced at least 10-fold during Ad2 infection (8). Therefore, it was another candidate for an antigen to be recompartmentalized during Ad2 infection. An ANA to topoisomerase I (AF/CDC9, anti-Scl-70) also indicated that topoisomerase I is recompartmentalized during Ad2 infection (Fig. 5); this ANA signal is diffuse in the nuclei in uninfected cells, and the diffuse pattern remains in Ad2-infected cells, but foci corresponding to MVM TP foci are also evident. An ANA which recognizes a centromere antigen (AF/CDC8) exhibits a speckled pattern in uninfected HeLa cells (Fig. 5); in Ad2-infected HeLa cells, that pattern is unchanged and clearly distinguishable from the MVM TP foci (Fig. 5), again indicating that the replication factory pattern is not the result of nonspecific protein aggregation.

Therefore, our data suggest a selective but coordinated relocalization of nucleolar machinery by Ad2 to form pseudonucleoli which contain several nucleolar proteins and snRNP antigens. The change in intranuclear compartmentalization of nucleolar protein B23 by adenovirus may be similar to B23 changes during tumorigenesis; the synthesis of protein B23 (numatrin) is induced by mitogens and is elevated in several lymphoma cell lines (14, 15), while B23 is recompartmentalized from the nucleoli to the nucleoplasm by antitumor agents which inhibit rRNA synthesis (59).

MVM-adenovirus interactions. Adenovirus can also serve as a helper virus for parvoviruses (3). We have found that Ad2 can help MVM DNA replication in HeLa cells in that MVM DNA replicates very poorly in HeLa cells, but the amount of MVM DNA that is replicated in HeLa cells can be significantly increased by coinfection with Ad2 (E. Fox, P. T. Moen, and J. W. Bodnar; manuscript in preparation). However, these MVM infections are nonproductive since no infectious MVM virions are produced as determined by plaque assays of the HeLa supernatants on mouse A9 cells.

In HeLa cells, adenovirus not only helps MVM DNA replication but recompartmentalizes it as well. When HeLa cells were infected with MVM alone, MVM DNA could be detected in situ, and the MVM DNA was localized as before in the host nucleoli (Fig. 6). In coinfections with Ad2 (Fig. 6), MVM replicated outside the nucleoli in multiple foci similar to the replication factories in number, size, and distribution.

The data are consistent with a mechanism by which MVM targets the host nucleolus for its replication and, when it is helped by adenovirus in HeLa cells, targets the pseudonucleoli induced by adenovirus. Our data suggest that these foci may contain components necessary for adenovirus replication and that MVM merely uses these new targets of opportunity for its own replication.

DISCUSSION

We have studied the interactions of MVM and adenovirus with host nucleoli. Our study has concentrated on intranuclear compartmentalization of MVM and Ad2 DNA and host nucleolar antigens and has indicated specific ways that these viruses interact with nucleoli. The combined data suggest several more general aspects of host-virus and virus-helper virus interactions.

Two different genera of viruses specifically interact with the host nucleoli during lytic infection. MVM uses the nucleoli directly, while adenovirus degrades the nucleoli and induces pseudonucleoli throughout the nucleus. Colocalization experiments with Ad5 DBP, which has been shown previously to compartmentalize to Ad5 DNA replication factories (56), indicate that several nucleolar and snRNP antigens are coordinately and specifically recompartmentalized to the replication factories during Ad2 infection. While we still need to show conclusively that the Ad2 replication factories and MVM DNA-containing foci are identical, their size, numbers, and patterns in Ad2-infected HeLa cells are identical, suggesting that the Ad2 replication factories contain nucleolar components, including MVM TP, and are the sites of MVM DNA replication.

The interactions with nucleoli may be common to more genera of mammalian DNA viruses. For example, host nucleoli show an early hypertrophy (19) and simian virus 40 DNA is associated with the nucleoli during simian virus 40 infections (18), while rRNA genes can be activated by either

simian virus 40 infection or simian virus 40 T antigen in vitro (50). In herpesvirus infections, the nucleoli are degraded (28, 47) and rRNA synthesis is inhibited (28, 57).

Adenovirus infection induces coordinated recompartmentalization of nucleolar components and snRNPs. We have found seven separate nucleolar components that are recompartmentalized during adenovirus infection (AgNOR-staining nucleolar components; proteins B23, NOR90, polymerase I, AF/CDC6, and MVM TP; and MVM DNA). Adenovirus replication factories have already been shown to be the intranuclear sites of the Ad2-encoded DNA polymerase (42), the Ad5 72-kDa DBP (56), and the Ad2 33-kDa late nonstructural protein (17). Also, the E1A proteins have been shown to be localized in several patterns including nucleolar and punctate (similar to replication factories); the E1A proteins colocalize in Ad2-infected cells with the heat shock protein hsp70, which is found in nucleoli of heat-shocked uninfected cells (13, 58). We suggest that adenovirus coordinately relocalizes appropriate nucleolar gene products to form pseudonucleoli nucleated around the input adenovirus DNA, which then serve as sites for adenovirus DNA replication (although we cannot currently discount that the nucleolar proteins are merely binding nonspecifically to the Ad2 DNA or chromatin). We suggest that this effect is mediated by the E1A/E1B genes since 293 cells (Ad5-transformed human fibroblasts) can support MVM DNA replication without an additional adenovirus helper (Fox et al., in preparation).

The splicing snRNP antigens U1 and Sm are recompartmentalized to replication factories during Ad2 infection, while the snRNP antigens Th, U2, U3, and Fi (L5/5S) are not. This may represent recompartmentalization of specific splicing machinery required for processing of Ad2 mRNAs.

Parvoviruses can parasitize helper viruses by utilizing pseudonucleoli formed by the helper viruses. Parvovirus replication often depends on a second helper virus, which is usually adenovirus or a herpesvirus (3). The recompartmentalization of MVM DNA in Ad2-infected HeLa cells suggests that this property of the parvoviruses depends on the ability of the helper adenovirus to provide an intranuclear compartment for replication which contains nucleolar components, presumably including nucleolar replication machinery. This mechanism may also apply to the herpesviruses since they (i) can help adeno-associated virus DNA replication (3); (ii) replicate their DNA in multiple intranuclear foci which contain the viral DBP ICP8 (26, 27); (iii) degrade host nucleoli and inhibit rRNA synthesis (28, 57); and (iv) induce and recompartmentalize AgNOR-staining components and B23 in HeLa cells (data not shown).

Nucleolar interaction may be a mechanism common to oncogenesis and to the lytic growth of oncogenic and oncolytic viruses. Aberrant nucleolar morphology or increased synthesis of nucleolar antigens is common in tumor cells, and nucleolar changes may be diagnostic of the transformed phenotype (6, 16). Adenoviruses are oncogenic in that they can induce tumors in nonpermissive rodent hosts (54). On the other hand, parvoviruses are "oncolytic" in that they grow preferentially in tumor cells in rodents and can suppress transformation of tissue culture cells by several carcinogens and oncogenic viruses (10). We suggest that the nucleolar changes induced by adenovirus and parasitized by MVM may be similar to those induced during tumorigenesis.

In conclusion, we have begun to identify specific biochemical ways by which MVM and adenovirus interact with the nucleolus for their lytic growth. By studying MVM and adenovirus both in normal hosts and in a host in which adenovirus helps and recompartmentalizes MVM DNA replication, we are beginning to see the role of intranuclear compartmentalization in the MVM interactions with both its host and helper viruses as well as in the oncolytic property of parvoviruses.

ACKNOWLEDGMENTS

We thank Katherine McHenry for technical assistance. We thank David Ward of Yale University, Marie Chow of Massachusetts Institute of Technology, and George Pearson of Oregon State University for providing cells and viruses. We thank P. K. Chan and Harris Busch of Baylor College of Medicine, Mark Olson of the University of Mississippi, Vaughn Cleghon and Daniel Klessig of the Waksman Institute, and Susan Cotmore, Peter Tattersall, Susan Baserga, and Joan Steitz of Yale University for providing antisera.

This work was supported by Public Health Service grant GM-35238 from the National Institutes of Health.

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