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Organization of Cytoskeleton Elements during Herpes Simplex Virus Type 1 Infection of Human Fibroblasts: An Immunofluorescence Study

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SUMMARY

Cultured human fibroblasts showed a typical fibrillar organization of microtubules in immunofluorescence, including the vimentin type of intermediate filament as well as actin-containing microfilaments. During infection with herpes simplex virus type 1 (HSV-1), the vimentin organization was maintained whereas actin, myosin and tubulin showed a progressive association with the viral glycoproteins within juxtanuclear structures. These structures could also be revealed with fluorochrome-coupled wheat germ agglutinin. Disruption of the microtubules by demecolcine treatment or their stabilization by taxol treatment did not prevent the aggregation of viral proteins in the cytoplasm. Taxol stabilization of the microtubules allowed the juxtanuclear accumulation of the glycoproteins in HSV-infected cells whereas treatment with demecolcine led to an accumulation of the glycoproteins either in small vesicles in the cytoplasm or in the focal adhesion areas of the cells. Production of infectious intracellular virus particles was reduced in cells treated with demecolcine or with taxol before and during infection. The results of this study indicate that the normal intracellular transport and distribution of the HSV glycoproteins and the formation of infectious virus are dependent on the presence of intact microtubules.

INTRODUCTION

The cytoplasmic organization of cultured cells has been studied extensively and the fibrillar cytoskeletal components (microfilaments, microtubules and intermediate filaments) have been defined by fluorescent antibody staining (Weber & Osborn, 1981; Pollard *et al.*, 1976). The actin-containing microfilaments are important for cell morphology, and the microfilaments are associated with the plasma membrane of the cells (Tilney, 1983; Pollard *et al.*, 1976; Weihing, 1979). The microtubules are involved in organelle movement and probably also in cellular secretory phenomena (Schliwa, 1984; for review, see Dustin, 1978). Much less is known about the function of intermediate filaments, but they may mediate the anchorage of the cell nuclei and play a role in the maintenance of cell shape (Lehto *et al.*, 1978; Menko *et al.*, 1983; for reviews, see Osborn *et al.*, 1981; Virtanen *et al.*, 1981).

Cell morphology and the integrity of the cytoskeleton components are often changed by viral infections. For instance, infection with RNA viruses leads to changes in the number of microfilaments (Rutter & Mannweiler, 1977; Fagraeus *et al.*, 1978; Bedows & Welsh, 1983), and the paramyxovirus M protein apparently interacts with actin filaments through direct binding (Giuffrè *et al.*, 1982). Actin filaments are also re-organized in certain cells after infection with DNA viruses such as vaccinia virus (Meyer *et al.*, 1981; Hiller *et al.*, 1981). Changes in the organization of intermediate filaments have been demonstrated for reovirus-, frog virus 3- and canine distemper virus-infected cells (Sharpe *et al.*, 1982; Murti & Goorha, 1983; Howard *et al.*, 1983).

The morphogenesis of herpes simplex virus (HSV) is not well understood. It seems to be much more complex than the morphogenesis of other enveloped viruses. Although information about the role of cytoskeleton elements in viral morphogenesis is accumulating steadily, most of these

results are probably not directly applicable to the HSV maturation process. Changes in the structure of actin filaments have been observed in HSV-infected cells (Bedows & Welsh, 1983; Winkler *et al.*, 1982; Heeg *et al.*, 1981), and cytoskeleton-disrupting drugs have also been used in the analysis of HSV morphogenesis (Dix & Courtney, 1976; Heeg *et al.*, 1981).

In the present study, we show that HSV infection of human fibroblasts leads to reorganization of both actin filaments and microtubules. We also present results which support the hypothesis that the viral glycoproteins interact with cytoskeletal elements during intracellular transport, and intact microtubules are shown to be important for the formation of infectious virus.

METHODS

Infection of cells. Human fibroblasts were cultured on coverslips in Leighton tubes in RPMI 1640 medium with 10% foetal calf serum (FCS) (Gibco). Cells were infected with HSV-1 (strain F) at a m.o.i. of 10 p.f.u./cell in RPMI 1640 medium with 1% FCS. The HSV-1 (F) prototype strain was obtained from Dr B. Roizman (University of Chicago, Chicago, Ill., U.S.A.). For certain experiments cells were exposed to 10 µl/ml taxol (Ciba, Milan, Italy) by use of a stock solution at 10 mg/ml in dimethyl sulphoxide (DeBrabander *et al.*, 1981). The treatment was done as specified in Results. Treatment of the cells with 1 µg/ml demecolcine was done as specified below, by use of a stock solution at 2 mM in distilled water.

Preparation of HSV-1-infected cell extract for immunoblotting. Monolayer cells grown in 75 cm² flasks and infected as described above were labelled with 20 µCi/ml [³⁵S]methionine from 7 to 9 h post-infection (sp. act. 1098 Ci/mmol, New England Nuclear), and at 9 h post-infection were extracted for 30 min at 22 °C in 5 ml 40 mM-Tris-HCl, 2 mM-EDTA buffer pH 7.4, supplemented with 0.5% Triton X-100 and 10⁻⁵ M of both TPCK and TLCK (Lehto *et al.*, 1978). After one wash in 5 ml of the same buffer, the attached infected cell proteins (ICPs) were removed and solubilized in 1.5 ml of a disruption mixture containing 2% (w/w) SDS and 5% (v/v) 2-mercaptoethanol (Morse *et al.*, 1978). The extract was separated in SDS-polyacrylamide gels as detailed by Morse *et al.* (1978), and transferred to nitrocellulose membranes (Braun *et al.*, 1983).

Antibodies. Monoclonal antibodies to the HSV-1 glycoproteins gB (antibody H-233), gC (H-C-1) and gD (H-D-1) were obtained from Dr L. Pereira (California Department of Health Services, Berkeley, Ca., U.S.A.). The specificities of the antibodies are detailed elsewhere (Pereira *et al.*, 1980). Rabbit antibodies to gB, gC and gD were produced by immunization with immunoprecipitates (Vestergaard & Norrild, 1979), and hyperimmune reference serum to the HSV-1 proteins was made by infection of rabbits (Norrild & Vestergaard, 1977). The immunoglobulin fraction was purified from these and from preimmune rabbit sera (Harboe & Ingild, 1973). Rabbit antibodies to actin, myosin, tubulin and vimentin have been characterized previously (Badley *et al.*, 1978; Virtanen *et al.*, 1981). The rabbit antibodies to actin, myosin and tubulin were kindly provided by Dr R. A. Badley (Unilever Research, Bedford, U.K.). Monoclonal antibodies to α tubulin were obtained from Amersham. In immunoblotting of uninfected cultured fibroblast extracts, these antibodies bind only to actin, myosin, tubulin and vimentin respectively (Virtanen *et al.*, 1984). The specificity of the cytoskeleton antibodies was tested also in immunoblotting of HSV-1-infected fibroblast extracts prepared from cells harvested 9 h post-infection (Fig. 5). The monoclonal antibodies were diluted 1:500 and the rabbit antibodies were diluted 1:50. The incubation was done for 18 h at 37 °C. Peroxidase-coupled swine anti-rabbit serum (no. P217) was purchased from Dakopatts (Copenhagen, Denmark) and peroxidase-coupled goat anti-mouse serum from Tago (Burlingame, Ca., U.S.A.). The substrate was 1-chloro-4-naphthol.

Indirect immunofluorescence (IIF). Infected and uninfected cells were fixed at the times after infection indicated in the figure legends. The fixation was carried out by treatment with cold methanol at -20 °C for 15 min or with 3% paraformaldehyde at room temperature for 15 min. The fixed cells were stored in phosphate-buffered saline with 0.1% sodium azide at 4 °C until use. Staining with either rabbit or monoclonal antibodies was done as described previously (Norrild *et al.*, 1983a). For single staining, fluorescein isothiocyanate (FITC)-coupled conjugates were used. For rabbit sera a swine anti-rabbit conjugate (no. F205; Dakopatts) and for monoclonal antibodies a goat anti-mouse conjugate (Cappel Laboratories) were used. In double-staining experiments the goat anti-mouse and the swine anti-rabbit immunoglobulins were either rhodamine (TRITC)- or FITC-coupled as specified in the figure legends. TRITC-coupled goat anti-mouse immunoglobulin was purchased from Cappel Laboratories and TRITC-coupled swine anti-rabbit immunoglobulin was from Dakopatts (no. R156). The coverslips were embedded in sodium barbital/glycerol buffer pH 8.4 and examined in a Zeiss Universal microscope equipped with filters for FITC and TRITC fluorescence.

Titration of infectious virus particles. HSV-1-infected fibroblasts grown in the presence of either taxol or demecolcine were harvested 9 h post-infection. Intracellularly produced virus was quantified in a standard plaque assay done on monolayers of Vero cells (Roizman & Roane, 1961). The virus production was compared to that of untreated infected cells.

Table 1. *Infectious HSV-1 particles made intracellularly during taxol or demecolcine treatment*

	P.f.u./2 × 10 ⁶ cells*	Inhibition (%)
Untreated		
HSV-1-infected		
9 h post-infection	1.69 × 10 ⁸	0
Taxol treatment		
- 12 h to 9 h post-infection	9.07 × 10 ⁷	46.3
Demecolcine treatment		
- 1 h to 9 h post-infection	1.63 × 10 ⁷	90.4

* Mean value of two experiments.

RESULTS

Indirect immunofluorescence staining with rabbit antibodies made to actin, myosin, tubulin and vimentin (Fig. 1) showed a typical organization of microfilaments, intermediate filaments and microtubules in uninfected human embryonic fibroblasts. The pattern of IIF staining of microfilaments with antibodies to actin overlapped that obtained with antibodies to myosin and therefore is not shown. In HSV-1-infected cells the organization of vimentin filaments appeared normal whereas both microfilaments and tubulin appeared to be located in juxtanuclear aggregates which were seen as early as 6 h post-infection but were more pronounced later in the infectious cycle. The juxtanuclear structures could also be stained with antibodies to the HSV glycoproteins as demonstrated by first staining the infected cells with rabbit antibodies to the glycoproteins gB, gC or gD and then staining with monoclonal antibodies to tubulin. The results obtained with antibodies to gD are presented in Fig. 2. Preimmune serum did not stain the infected cell proteins (data not shown). As shown earlier (Norrild *et al.*, 1983*a*), the juxtanuclear structure apparently corresponds to the Golgi apparatus in infected cells.

Treatment of the HSV-1-infected fibroblasts with taxol, a drug which is known to stabilize microtubules (DeBrabander *et al.*, 1981; Thompson *et al.*, 1981), for 12 h before infection and maintenance of the infected cells in taxol-containing medium allowed the accumulation of the glycoproteins in the juxtanuclear aggregates (Fig. 3*b*). In these cells a pattern typical of re-aggregated tubulin was seen (Fig. 3*a*), and it should be noted that the glycoprotein-containing vesicles were surrounded by re-organized tubulin bundles which apparently stretched into the cytoplasmic compartment occupied by the vesicles. Taxol treatment prevented insertion of the viral glycoproteins into the plasma membrane of infected cells as indicated by the lack of membrane fluorescence of unpermeabilized, paraformaldehyde-fixed cells stained with antibodies to the various glycoproteins (Fig. 3*c*). Pretreatment of the cells with demecolcine for 1 h before infection led to a complete disruption of microtubules (Virtanen *et al.*, 1980*b*) as well as a complete disruption of the Golgi apparatus (Virtanen *et al.*, 1980*a*), and the viral glycoproteins appeared in vesicles or aggregates which accumulated close to the periphery of the cell, although the cells did not show membrane fluorescence (Fig. 4). Identical results were obtained with antibodies to gB, gC and gD, although only staining with antibodies to gD is shown in the figures.

The production of infectious virus in cells pretreated and maintained with either taxol or demecolcine was analysed. The virus titres as measured by plaque formation were reduced by 46% after taxol treatment but by 90% in demecolcine-treated cells (Table 1).

Association between the cytoskeleton elements and the glycoprotein-containing vesicles could not be demonstrated after extraction of cultured human fibroblasts with 0.5% Triton X-100, which has previously been shown to produce cytoskeleton preparations that adhere to the substratum. The extraction leaves the microfilaments, intermediate filaments and microtubules in a near-normal organization (Lehto *et al.*, 1978; Osborn & Weber, 1977). Extraction of HSV-1-infected fibroblasts showed that the cytoskeleton adhered to the growth substratum as shown by IIF staining with antibodies to vimentin, tubulin and actin, but the juxtanuclear viral structures visible by IIF staining of intact fixed cells disappeared after the extraction (data not shown).

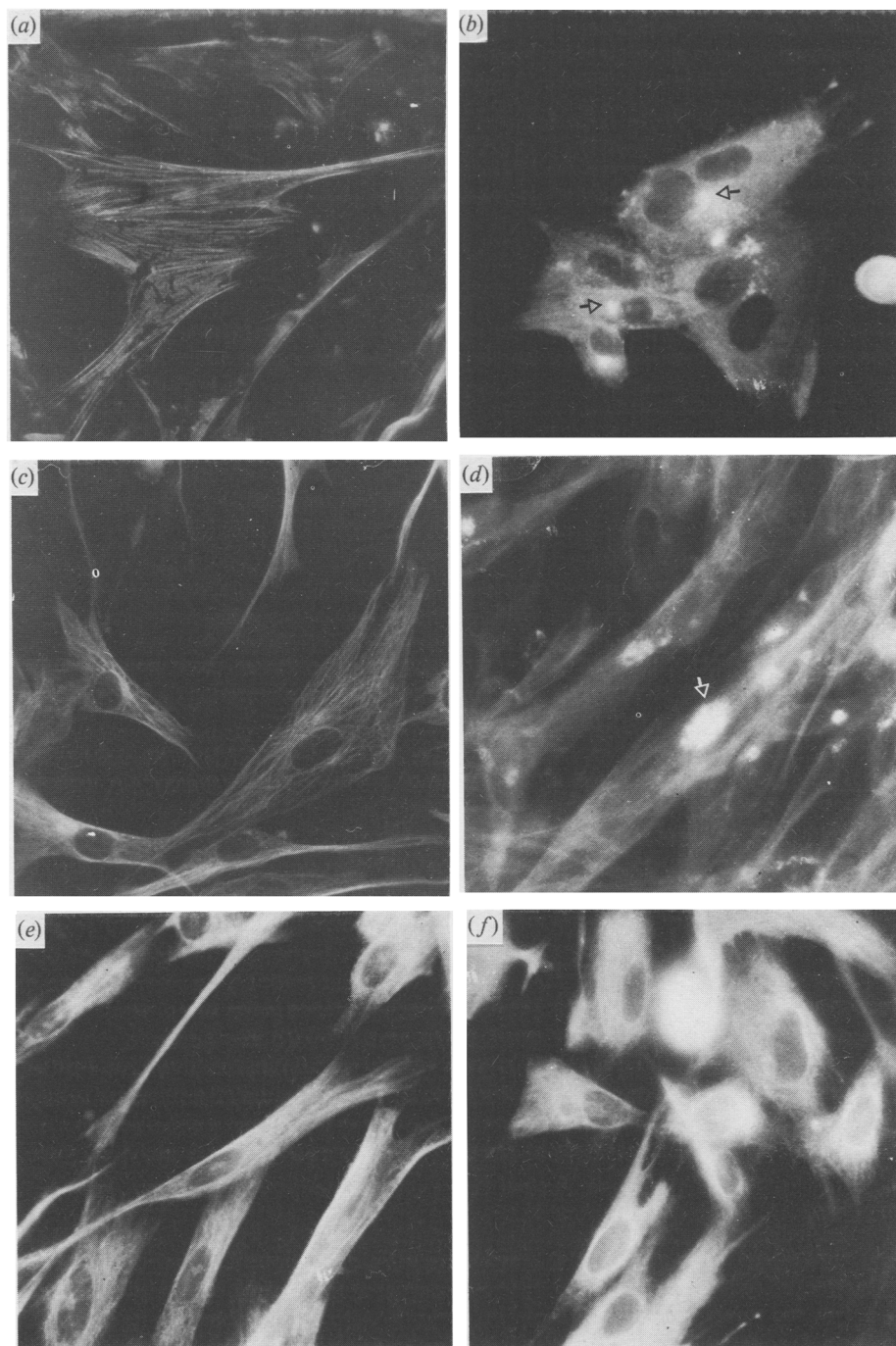


Fig. 1. Cytoskeletal fibrillar structures of uninfected (*a, c, e*) and HSV-1-infected (*b, d, f*) cultured human fibroblasts 9 h post-infection. Cells were fixed in methanol and stained for IIF with rabbit antibodies to myosin (*a, b*), tubulin (*c, d*) or vimentin (*e, f*) and FITC-coupled swine anti-rabbit immunoglobulin. Note the aggregation of myosin and tubulin in HSV-1-infected cells (*b, d*) (arrows). Magnification $\times 320$.

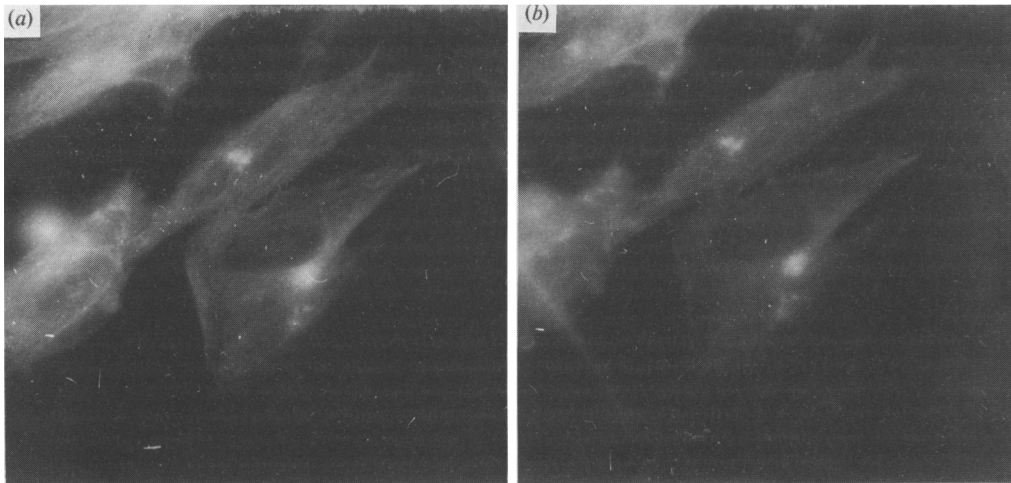


Fig. 2. Intracellular location of tubulin and HSV-1 gD in infected human fibroblasts 9 h post-infection. Cells were fixed in methanol and double-stained for IIF with monoclonal antibodies to tubulin (a) and rabbit monospecific antibodies to gD (b). TRITC-coupled goat anti-mouse antibodies and FITC-coupled swine anti-rabbit antibodies were used. Note the association of tubulin and gD aggregates in (a). Magnification $\times 360$.

Extracts of cytoskeleton proteins from HSV-1-infected cells were also analysed for HSV-associated proteins by immunoblotting. The transferred proteins were incubated with antibodies to vimentin, tubulin, actin or HSV proteins (Fig. 5). None of the viral glycoproteins was present in the extract. Only ICPs 5, 8, 24 and 36 were present in quantities which allowed a strong reaction with the hyperimmune HSV-1 serum (Fig. 5d).

DISCUSSION

The HSV-1 glycoproteins synthesized during infection of cultured cells are present in perinuclear structures and in the nuclear membrane early in the infectious cycle (Wenske *et al.*, 1982; Norrild *et al.*, 1983a). The glycoproteins are processed through the Golgi apparatus and they accumulate together with immature virions in Golgi-derived vesicles in monensin-treated cells (Norrild *et al.*, 1983a; Johnson & Spear, 1982; Wenske *et al.*, 1982). The mode of transport of individual glycoproteins from the Golgi apparatus to the plasma membrane is mostly unknown but it apparently involves various routes. Glycoprotein D is transported to the vinculin-containing adhesion plaques (Norrild *et al.*, 1983b; Geiger, 1981), and the three glycoproteins gB, gC and gD were transported to and inserted into the plasma membrane including the cell-cell junction areas (Norrild *et al.*, 1983b).

In HSV-infected cells the significance of the cytoskeleton elements in the transport of the HSV glycoproteins has not been studied previously. The drug cytochalasin, which prevents the glycosylation of the viral proteins, also inhibits the formation of infectious virus. Whether this is an effect of the cytochalasin-induced disruption of the microfilaments is unknown (Dix & Courtney, 1976). The HSV-1 capsid protein ICP-5 and the major DNA-binding protein ICP-8 are associated with the cellular cytoskeleton complex (Quinlan & Knipe, 1983; Ben-Ze'ev *et al.*, 1983) and our results show that ICP-24 and ICP-36 are attached to the fibrillar network.

The present study demonstrates that HSV infection of human fibroblasts led to profound changes in the organization of both actin- and myosin-containing microfilaments and microtubules as early as 6 h post-infection, whereas the intermediate filaments remained intact. Actin, myosin and tubulin aggregated in juxtanuclear structures which also stained with antibodies to the viral glycoproteins. This apparent viral protein 'factory' was therefore either

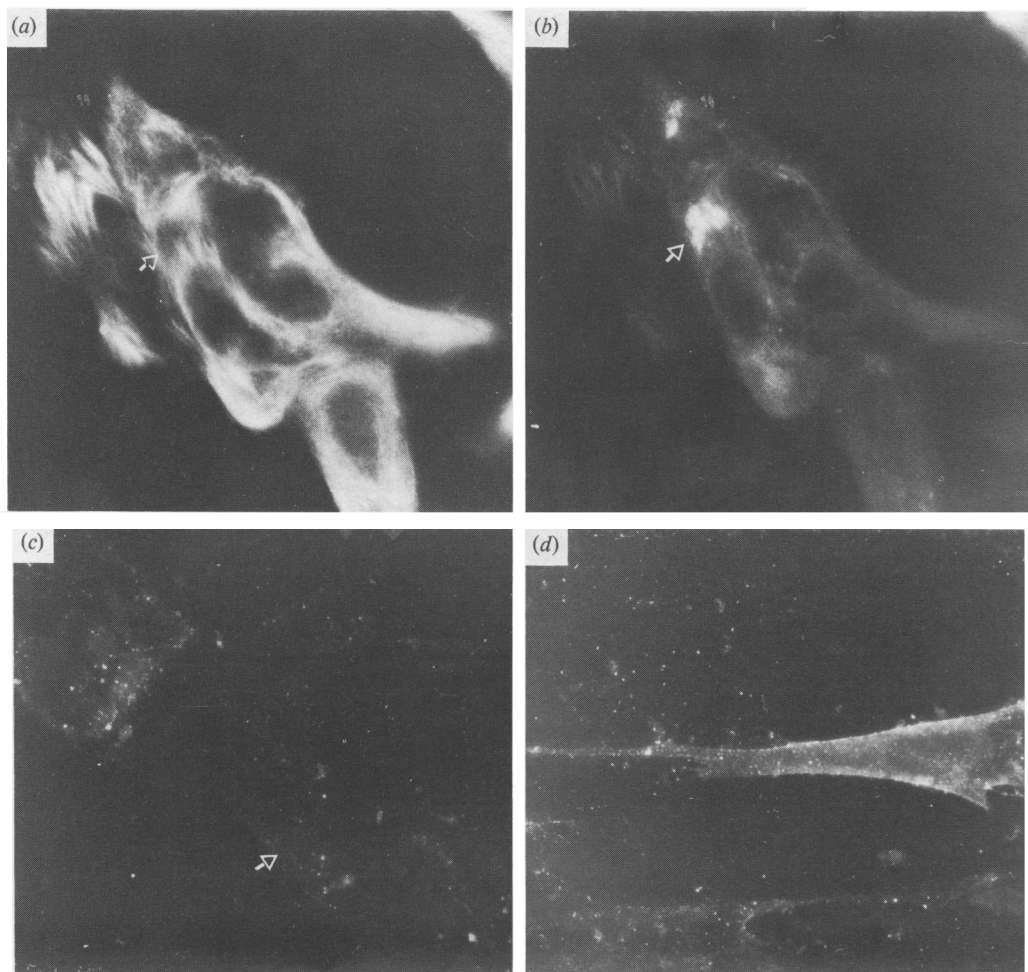


Fig. 3. Intracellular location of tubulin and HSV-1 gD in infected human fibroblasts exposed to taxol. Human cultured fibroblasts were exposed to taxol 12 h before infection, during infection and after infection until the cells were fixed at 9 h post-infection. Methanol-fixed cells were double-stained for IIF with rabbit anti-tubulin antibodies (a) and monoclonal antibodies to gD (b). Paraformaldehyde-fixed HSV-1-infected cells treated with taxol were stained with monoclonal antibodies to gD (c). Paraformaldehyde-fixed HSV-1-infected untreated cells were stained with monoclonal antibodies to gD (d). TRITC-coupled anti-rabbit and FITC-coupled anti-mouse antibodies were used. The characteristic organization of the reassembled microtubules in taxol-pretreated cells can be seen in (a); the arrow indicates a bundle of microtubules which ends where the glycoprotein-containing vesicles accumulate in the cell. Note the accumulation of gD (arrow in b). A lack of membrane staining in (c), and the positive membrane staining in (d) can be seen. Magnification $\times 360$.

formed in association with microfilaments and/or microtubules, or it had trapped cytoskeletal proteins from a pool of unpolymerized proteins. Treatment of HSV-infected cells with taxol or with demecolcine did not prevent accumulation of the viral glycoproteins but only affected their integration into the plasma membrane. In taxol-treated cells the glycoprotein-containing vesicles were observed at the tip of certain tubulin bundles, an observation which was also made in vesicular stomatitis virus-infected cells, where a direct association between the Golgi apparatus and the microtubules was demonstrated (Rogalski & Singer, 1984).

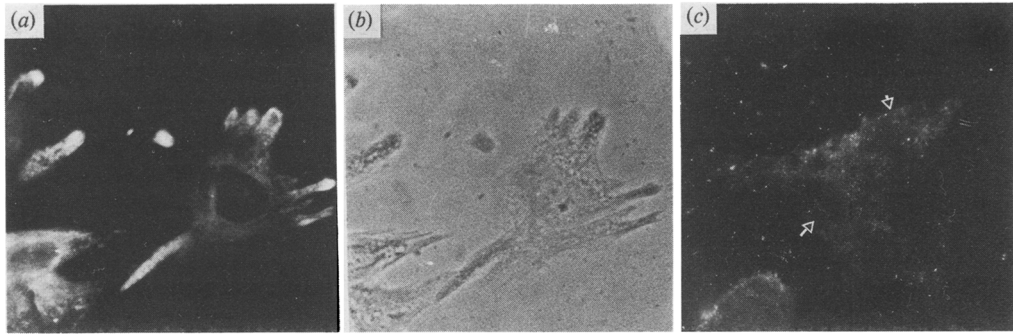


Fig. 4. Intracellular location of gD in HSV-1-infected demecolcine-treated cells. Cells were exposed to the drug for 1 h before infection, during infection and until fixation at 9 h post-infection. IIF staining of methanol-fixed cells stained with monoclonal antibodies to gD and FITC-coupled goat anti-mouse antibodies is shown in (a). Note the gD-containing vesicles in the cytoplasm of the cells. (b) Phase-contrast microscopy of the same cell. (c) Paraformaldehyde-fixed cells stained for gD. A lack of membrane staining can be seen. Magnification $\times 260$.

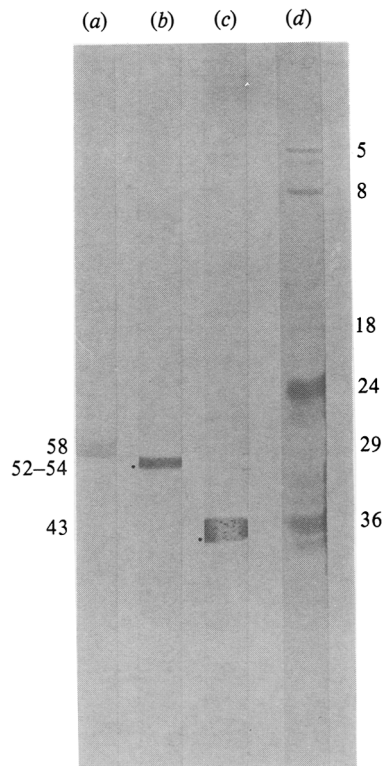


Fig. 5. Immunoblotting of Triton X-100-insoluble HSV-1 ICPs reacted with monoclonal antibodies to (a) vimentin and (b) tubulin, and (c) rabbit antibodies to actin. (d) Immunoblotting with rabbit hyperimmune reference serum made to HSV-1. The HSV-1 insoluble proteins were identified on the autoradiogram made by exposure of the transferred proteins (not shown) and the ICP numbers as identified by Morse *et al.* (1978) are listed at the right. The molecular weights as estimated by the electrophoretic mobility of vimentin, tubulin and actin are marked at the left. Note the lack of immunological cross-reactivity between the antibodies made to the proteins of the cytoskeletal fibrillar structures and the HSV-1 proteins.

The complete re-distribution of HSV glycoproteins when the Golgi apparatus is dispersed and the vimentin filaments are re-organized after the addition of demecolcine indicates that the transport and sorting of the glycoprotein-containing structures is dependent on intact cytoskeleton fibrillar components. Disruption of microtubules led to a changed membrane distribution of the G glycoprotein of vesicular stomatitis virus but the protein was inserted into the membrane (Rogalski *et al.*, 1984).

As infectious HSV is still produced and released to the surrounding medium from demecolcine-treated cells, although in reduced amounts, our observations indicate that transport of HSV glycoproteins and particles may follow different routes in infected cells. Before we can understand HSV morphogenesis in detail, further analysis of the interaction between the cellular and the viral structures will be necessary.

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REFERENCES

- BADLEY, R. A., LLOYD, C. W., WOODS, A., CARRUTHERS, L., ALLCOCK, C. & REES, D. A. (1978). Mechanisms of cellular adhesion. III. Preparation and preliminary characterization of adhesion. *Experimental Cell Research* **117**, 231–244.
- BEDOWS, E. & WELSH, M. J. (1983). Fate of microfilaments in Vero cells infected with measles virus and herpes simplex virus type 1. *Molecular and Cellular Biology* **3**, 712–719.
- BEN-ZE'EV, A., ABULAFIA, R. & BRATOSIN, S. (1983). Herpes simplex virus and protein transport are associated with the cytoskeletal framework and the nuclear matrix in infected BSC-1 cells. *Virology* **129**, 501–507.
- BRAUN, D. K., PEREIRA, L., NORRILD, B. & ROIZMAN, B. (1983). Application of denatured, electrophoretically separated, and immobilized lysate of herpes simplex virus-infected cells for detection of monoclonal antibodies and for studies of the properties of viral proteins. *Journal of Virology* **46**, 103–112.
- DEBRABANDER, M., GEUENS, G., NUYDENS, R., WILLEBRORDS, R. & DEMAY, J. (1981). Taxol induces the assembly of free microtubules in living cells and blocks the organizing capacity of the centrosomes and kinetochores. *Proceedings of the National Academy of Sciences, U.S.A.* **78**, 5608–5612.
- DIX, R. D. & COURTNEY, R. J. (1976). Effects of cytochalasin B on herpes simplex virus type 1 replication. *Virology* **70**, 127–135.
- DUSTIN, P. (1978). *Microtubules*. Berlin, Heidelberg & New York: Springer-Verlag.
- FAGRAEUS, A., TYRRELL, D. L. J., NORBERG, R. & NORRBY, E. (1978). Actin filaments in paramyxovirus-infected human fibroblasts studied by indirect immunofluorescence. *Archives of Virology* **57**, 291–296.
- GEIGER, B. (1981). Involvement of vinculin in contact-induced cytoskeletal interactions. *Cold Spring Harbor Symposia on Quantitative Biology* **46**, 671–682.
- GIUFFRÉ, R. M., TOVELL, D. R., KAY, C. M. & TYRRELL, D. L. J. (1982). Evidence for an interaction between the membrane protein of a paramyxovirus and actin. *Journal of Virology* **42**, 963–968.
- HARBOE, N. & INGILD, A. (1973). Immunization, isolation of immunoglobulins, estimation of antibody titers. *Scandinavian Journal of Immunology* **2**, (supplement 1), 161–164.
- HEEG, U., HAASE, W., BRAUER, D. & FALKE, D. (1981). Microtubules and microfilaments in HSV-infected rabbit-kidney cells. *Archives of Virology* **70**, 233–246.
- HILLER, G., JUNGWIRTH, C. & WEBER, K. (1981). Fluorescence microscopical analysis of the life cycle of vaccinia virus in chick embryo fibroblasts. *Experimental Cell Research* **132**, 81–87.
- HOWARD, J. M., ECKERT, B. S. & BOURGUIGNON, L. Y. W. (1983). Comparison of cytoskeletal organization in canine distemper virus-infected and uninfected cells. *Journal of General Virology* **64**, 2379–2385.
- JOHNSON, D. C. & SPEAR, P. G. (1982). Monensin inhibits the processing of herpes simplex virus glycoproteins, their transport to the cell surface and the egress of virions from infected cells. *Journal of Virology* **43**, 1102–1112.
- LEHTO, V.-P., VIRTANEN, I. & KURKI, P. (1978). Intermediate filaments anchor the nuclei in nuclear monolayers of cultured fibroblasts. *Nature, London* **272**, 175–177.
- MENKO, A. S., TOYAMA, Y., BOETTIGER, D. & HOLZER, H. (1983). Altered cell spreading in cytochalasin B: a possible role for intermediate filaments. *Molecular and Cellular Biology* **3**, 113–125.
- MEYER, R. K., BURGER, M. M., TSCHANNEN, R. & SCHAFFER, R. (1981). Actin filament bundles in vaccinia virus infected fibroblasts. *Archives of Virology* **67**, 11–18.
- MORSE, L. S., PEREIRA, L., ROIZMAN, B. & SCHAFFER, P. A. (1978). Anatomy of herpes simplex virus (HSV) DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 × HSV-2 recombinants. *Journal of Virology* **26**, 389–410.
- MURTI, K. G. & GOORHA, R. (1983). Interaction of frog virus-3 with the cytoskeleton. I. Altered organization of microtubules, intermediate filaments, and microfilaments. *Journal of Cell Biology* **96**, 1248–1257.
- NORRILD, B. & VESTERGAARD, B. F. (1977). Polyacrylamide gel electrophoretic analysis of herpes simplex virus type 1 immunoprecipitates obtained by quantitative immunoelectrophoresis in antibody-containing agarose gel. *Journal of Virology* **22**, 113–117.

- NORRILD, B., VIRTANEN, I., PEDERSEN, B. & PEREIRA, L. (1983a). Requirements for transport of HSV-1 glycoproteins to the cell surface membrane of human fibroblasts and Vero cells. *Archives of Virology* **77**, 155–166.
- NORRILD, B., VIRTANEN, I., LEHTO, V.-P. & PEDERSEN, B. (1983b). Accumulation of herpes simplex virus type 1 glycoprotein D in adhesion areas of infected cells. *Journal of General Virology* **64**, 2499–2503.
- OSBORN, M. & WEBER, K. (1977). The detergent-resistant cytoskeleton of tissue culture cells includes the nucleus and the microfilament bundles. *Experimental Cell Research* **106**, 339–349.
- OSBORN, M., GEISLER, N., SHAW, G., SHARP, G. & WEBER, K. (1981). Intermediate filaments. *Cold Spring Harbor Symposia on Quantitative Biology* **46**, 413–429.
- PEREIRA, L., KLASSEN, T. & BARINGER, J. R. (1980). Type-common and type-specific monoclonal antibodies to herpes simplex virus type 1. *Infection and Immunity* **29**, 724–732.
- POLLARD, T. D., FUJIWARA, K., NIEDERMANN, R. & MAUPIN-SZAMIER, P. (1976). In *Cell Motility* (Cold Spring Harbor Conferences on Cell Proliferation, vol. 3), pp. 689–724. Edited by R. D. Goldman, T. D. Pollard & J. L. Rosenbaum. New York: Cold Spring Harbor Laboratory.
- QUINLAN, M. P. & KNIPE, D. M. (1983). Nuclear localization of herpesvirus proteins: potential role for the cellular framework. *Molecular and Cellular Biology* **3**, 315–324.
- ROGALSKI, A. A. & SINGER, S. J. (1984). Associations of elements of the Golgi apparatus with microtubules. *Journal of Cell Biology* **99**, 1092–1100.
- ROGALSKI, A. A., BERGMANN, J. E. & SINGER, S. J. (1984). Effect of microtubule assembly status on the intracellular processing and surface expression of an integral protein of the plasma membrane. *Journal of Cell Biology* **99**, 1101–1109.
- ROIZMAN, B. & ROANE, P. R., JR (1961). Studies of the determinant antigens of viable cells. I. A method, and its application in tissue culture studies, for enumeration of killed cells, based on the failure of virus multiplication following injury by cytotoxic antibody and complement. *Journal of Immunology* **87**, 714–727.
- RUTTER, G. & MANNWEILER, K. (1977). Alterations of actin-containing structures in BHK21 cells infected with Newcastle disease virus and vesicular stomatitis virus. *Journal of General Virology* **37**, 233–242.
- SCHLIWA, M. (1984). Mechanisms of intracellular organelle transport. In *Cell and Muscle Motility*, vol. 5, pp. 1–81. Edited by R. W. Dowben & J. W. Shay. New York: Plenum Press.
- SHARPE, A. H., CHEN, L. B. & FIELDS, B. N. (1982). The interaction of mammalian reoviruses with the cytoskeleton of monkey kidney CV-1 cells. *Virology* **120**, 399–411.
- THOMPSON, W. C., WILSON, L. & PURICH, D. L. (1981). Taxol induces microtubule assembly at low temperature. *Cell Motility* **1**, 445–454.
- TILNEY, L. G. (1983). Interaction between actin filaments and membranes gives spatial organization to cells. *Modern Cell Biology* **2**, 163–199.
- VESTERGAARD, B. F. & NORRILD, B. (1979). Crossed immunoelectrophoretic analysis and viral neutralizing activity of five monospecific antisera against five different herpes virus glycoproteins. In *Oncogenesis and Herpesviruses III*, pp. 243–248. Edited by G. de Thè, W. Henle & F. Rapp. Lyon: IARC Scientific Publications No. 24.
- VIRTANEN, I., EKBLOM, P. & LAURILA, P. (1980a). Subcellular compartmentalization of saccharide moieties in cultured normal and malignant cells. *Journal of Cell Biology* **85**, 429–434.
- VIRTANEN, I., LEHTO, V.-P., LEHTONEN, E. & BADLEY, R. A. (1980b). Organization of intermediate filaments in cultured fibroblasts upon disruption of microtubules by cold treatment. *European Journal of Cell Biology* **23**, 80–84.
- VIRTANEN, I., LEHTO, V.-P., LEHTONEN, E., VARTIO, T., STENMAN, S., KURKI, P., WAGNER, O., SMALL, J. V., DAHL, D. & BADLEY, R. A. (1981). Expression of intermediate filaments in cultured cells. *Journal of Cell Science* **50**, 45–63.
- VIRTANEN, I., BADLEY, R. A., PAASIVUO, R. & LEHTO, V.-P. (1984). Distinct cytoskeletal domains revealed in sperm cells. *Journal of Cell Biology* **99**.
- WEBER, K. & OSBORN, M. (1981). Microtubule and intermediate filament networks in cells viewed by immunofluorescence microscopy. In *Cytoskeletal Elements and Plasma Membrane Organization*, pp. 1–53. Edited by G. Poste & G. L. Nicolson. Amsterdam: Elsevier/North-Holland.
- WEIHING, R. R. (1979). The cytoskeleton and plasma membrane. In *Methods and Achievements in Experimental Pathology*, vol. 8, pp. 42–109. Edited by G. Jasmin & M. Cantin. Basel: S. Karger.
- WENSKE, E. A., BRATTON, M. W. & COURTNEY, R. J. (1982). Endo- β -N-acetylglucosaminidase H sensitivity of precursors to herpes simplex virus type 1 glycoproteins gB and gC. *Journal of Virology* **44**, 241–248.
- WINKLER, M., DAWSON, G. J., ELIZAN, T. S. & BERL, S. (1982). Distribution of actin and myosin in rat neuronal cell line infected with herpes simplex virus. *Archives of Virology* **72**, 95–103.

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