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THE ROLE OF CYTOSKELETON AND NUCLEAR MATRIX IN VIRUS REPLICATION

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Summary. — In the light of the cytoarchitecture concept, the presented review attempts to cover what is known of the involvement of cytoskeletal and nucleoskeletal elements in the replication cycle of various viruses and in cell transformation. Our knowledge on the relationship of virus replication with cell architecture has rapidly progressed during the recent years in association with studies on composition of various filaments within cells, their spatial organization and possible functions which focussed much interest on the cytoskeleton and nuclear matrix. The new results indicated that highly specialized elements for normal cell function may be coopted for virus growth. Recently, using various methodical approaches it has been possible to obtain information about the association of virus-structural proteins with cytoskeletal and nucleoskeletal elements, about the reorganization of cytoskeleton during virus replication, about the role of tubulin in transcription and RNA synthesis of negative-strand viruses, about the involvement of cytoskeletal filaments in the transport of viral proteins, virus penetration, virus assembly and release from the infected cell. The role of cytoskeleton in cell transformation and in initiation of DNA synthesis and intracellular signaling to cell proliferation has been also investigated.

Key words: cytoskeleton, nuclear matrix, virus replication, cell transformation

Introduction

The recent rapid progress in understanding the composition of various filaments within cells, their spatial organisation and possible functions has focused much attention on the cytoskeleton and nuclear matrix converting them into one of the central fields of interest in modern cell biology.

The term "cytoskeleton" and "nuclear matrix or nuclear skeleton" is commonly used to define several types of cytoplasmic or nuclear filaments. The elements such as microtubules, microtubule-associated proteins, microfilaments and intermediate filaments are major cytoskeleton components of

each cell. Microtubules are seen in electron micrographs as structures with a diameter of 25 nm and of varying lengths. In gross-section microtubules appear to have 5 nm thick walls. They are highly ordered polymers of the globular protein tubulin, which has a native molecular weight of about 110 kD being composed of two similar but not identical subunits with individual molecular weights of about 55 kD (Weatherbee, 1981; Cleveland, 1986). Microtubule-associated proteins (MAPs) are a class of proteins that copurify with the tubulin and maintain a constant stechiometry with the tubulin dimer during purification by assembly-disassembly (Weatherbee, 1981; Brinkley, 1982; Olmsted, 1986). Microfilaments are composed of subunits of the globular protein actin (42 kD), helically arranged in a double-stranded filament with a diameter of 4—7 nm. The polymerization of the globular actin monomers (G-actin) to filaments (F-actin) is usually accompanied by hydrolysis of ATP. Actin appears to be present in all eukaryotic cells and may be the most common cellular protein (Weatherbee, 1981; DeRosier and Tilney, 1984).

Intermediate filaments (IF) have dimensions between those of microtubules and microfilaments (intermediate-sized filaments or 10 nm filaments). IF are more insoluble than the majority of other cytoskeleton constituents (Steinert *et al.*, 1984; Geisler and Weber, 1986).

The study of the elements of cytoskeleton, their structure, the biochemistry of constituent proteins represent an extremely active area of research. In continuity with the cytoskeleton research, we have been witnesses, in the last few years, of an explosion of informations about the association of virus replication and assembly with the cytoskeleton elements. In the light of new findings on biochemical dissection of nuclei we record an extreme interest devoted to the association of viral proteins with nuclear matrix in productive infection as well as cell transformation. The nuclear matrix is represented by a residual structure that combines elements of three out of four nuclear regions, namely the pore lamina complex, a residual nucleolus and an internal fibrogranular nonchromatin network (Simard *et al.*, 1986; Kaufmann *et al.*, 1986; Dupuy-Coin *et al.*, 1986).

The nuclear lamina is a polymeric protein meshwork that lines the nucleoplasmic surface of the nuclear envelope. Recent work of Gerace (1986) demonstrates that the lamina is composed of intermediate-type filaments. The A, B and C lamina are the major proteins of the nuclear envelope. The complete nucleotide sequence of the coding region of the A and C lamina shows that these proteins are identical except for their carboxy termini. The most prominent structural feature of both lamina is an α-helical region of repeating heptads of amino acids that shows striking homology with the entire family of cytoplasmic intermediate filament proteins (McKeon *et al.*, 1986).

The association of virus replication and assembly with cytoplasmic and nuclear architecture may represent the use of elements in which highly specialized mechanisms for normal cell function have been coopted for virus growth. The study of cytoplasmic and nuclear organization encompasses

a large number of biological, biochemical and molecular biological methods. This highly organized architecture at all levels joins virus replication and assembly with a very large number of different steps and special methabolic pathways.

New data on the association of viral proteins with the cytoskeletal and nucleoskeletal framework are still fragmentary and no attempt has been done to prepare a comprehensive review.

Myxoviruses

Many studies indicate that microtubules play an important role in directing the movement of organelles (Murphy and Tilney, 1974; Schnapp *et al.*, 1985), secretion and transport of cytoplasmic vesicles involved in endocytosis and transcytosis (Redman *et al.*, 1975; Nagura *et al.*, 1979). Using various approaches, it has recently been possible to obtain more information about the synthesis and localization of influenza virus structural and nonstructural proteins in the host cell. Ultrastructural localization of the synthesis of virus-specific proteins such as haemagglutinin (HA) and neuraminidase (NA) in influenza virus-infected cells by peroxidase-labelled antibodies showed that positive peroxidase reaction appeared 4 hr post infection (p.i.) at the ribosomes of the rough endoplasmic reticulum (ER). The HA, however, did not accumulate in the cisternae of rough ER, but became peroxidase positive in the smooth ER and on the smooth membranes of small Golgi complex vesicles which migrate to the cell membrane (Čiampor *et al.*, 1974). A synchronized directed transfer of the envelope glycoproteins of the influenza and vesicular stomatitis viruses from the Golgi apparatus to the apical and basolateral surfaces, respectively, in Madin-Darby canine kidney cells can be achieved using temperature shift protocols (Rindler et al., 1985). Similarly, the smooth ER vesicles and post-Golgi vesicles containing viral envelope glycoproteins in guinea pig tongue-influenza virus infected cells are accompanied by numerous microtubular structures stretching to the apical surface where virus budding takes place (Čiampor, 1987).

The microtubule-depolymerizing agents colchicine and nocodazole as well as the microtubule assembly-promoting drug taxol were found to interfere with the normal polarized delivery and exclusive segregation of HA at the apical surface but not with the delivery and initial accumulation of G protein of vesicular stomatitis virus on the basolateral surface. The extent of HA segregation may depend on the dispersion of Golgi apparatus induced by the inhibitors or on the disruption of putative microtubules containing tracks that could direct vesicles from the trans Golgi apparatus to the cell surface. The vesicular stomatitis virus G protein is basolaterally segregated even when the Golgi elements are dispersed (Rindler *et al.*, 1987).

The influenza A virus polypeptides M, NP and HA are associated with the host cell cytoskeleton during vegetative infection. Even at the earliest stages of infection, when viral gene products are first detectable, the viral polypeptides are associated exclusively with the cytoskeleton. The cytoskeleton-bound influenza polypeptides do not represent for the most part

a cell-associated form of the assembled virus, since differing antigens are found located in different compartments of the cytoplasm including nucleus. Thus, virus assembly, while being possibly an important function of the cytoskeleton, may be only one functional aspect of the interaction between viral and host structural polypeptides. Influenza polypeptides are bound to the cytoskeleton by association with host cytoskeletal proteins. The finding that HA is resistant to release from the cytoskeleton by Triton detergent, which solubilizes membrane phospholipids, argues that cellular HA is not exclusively membrane-bound (Laevitt *et al.*, 1981).

It is known that reorganization of the cytoskeleton may change cell functions. Reorganization of microfilaments after infection of the cell with certain viruses or in transformed cells was studied with the aim to specify the role of cytoskeleton in virus infection (Celis, 1979; Graesmann *et al.*, 1980; Pautrat *et al.*, 1980; Meyer *et al.*, 1981; Bussereau and Perrin, 1982; Lösse *et al.*, 1982; Howard *et al.*, 1983; Kasamatsu *et al.*, 1983; Keski-Oja *et al.*, 1983; Murti and Goorha, 1983; Notter and Balduzi, 1984; Stanislawski *et al.*, 1984). The cytoskeleton isolated from influenza virus-treated plasma membranes contains more proteins and more actin than the cytoskeleton isolated from uninfected plasma membranes. Virus particles were found to be associated with this cytoskeleton, although the purified virus alone, treated in the same manner, did not sediment at low speed in the Triton X-100 insoluble residues. Autophosphorylation of cytoskeleton, especially of the polypeptides 24, 45, 65 and 105—110 kD was increased when cytoskeleton was prepared from virus-treated membranes. Electron microscopy of the Triton insoluble residue from virus-treated plasma membranes showed more structures similar to microfilament bundles than Triton insoluble residue from control membranes. Based on these results Križanová *et al.*, (1986) suggested that reorganization of membrane-associated cytoskeleton occured already at early stages of influenza virus infection. Actomyosin fibril fragments were found in influenza virus particles after bromelain treatment (Lozatulimowska *et al.*, 1981). The cellular actin has been identified in many enveloped viruses (Fleissner and Tress, 1973; Lamb *et al.*, 1976; Orvell, 1978; Tyrrell and Norrby, 1978; Wang *et al.*, 1976). Three hybridoma clones producing IgM antibodies against actin were obtained from mice immunized with purified paramyxovirus virions. The monoclonal antibodies evidently reacted with epitopes common to actin and vimentin (Fagraeus *et al.*, 1983).

Paramyxoviruses and Rhabdoviruses

Two different groups of negative-strand RNA viruses, the rhabdoviruses (vesicular stomatitis virus, VSV) and the paramyxoviruses (Sendai virus) share similar strategies for their reproduction (Kingsbury, 1977; Wagner, 1975). The pathway of biogenesis of VSV glycoprotein G has been well characterized and serves as a model for many viral and cellular integral membrane proteins attached to the plasma membrane. In VSV-infected cells, G protein is transported from the site of its synthesis in the rough ER and nuclear membranes to the site in the Golgi complex where its oligosaccharides are

modified. G protein is finally transported to the plasma membrane where it is incorporated into budding virions (Katz *et al.*, 1977; Morrison and Lodish, 1975; Puddington *et al.*, 1985; Tabas and Kornfeld, 1979; Knipe *et al.*, 1977). Puddington *et al.* (1986) underscore the importance of the amino acid sequence in the cytoplasmic domain for efficient transport of G protein to the cell surface. How are the cytoskeleton elements involved in the reproductive cycle of above mentioned viruses?

Tubulin acts as a positive transcription factor for in vitro RNA synthesis of two different negative-strand viruses: Sendai virus and VSV. The synthesis of both a leader-like RNA and the NP mRNA directed by detergent-disrupted purified Sendai virions was shown to be totally dependent on the addition of purified tubulin. The addition of purified tubulin, although not required, also stimulated 2- to 7-fold the mRNA synthesis directed by detergent-disrupted VSV virions. Observations of Moyer *et al.* (1986) suggest that tubulin has a primary role in the initiation of transcription directly at the 3'end of the Sendai virus genome. Microtubule-associated proteins (MAP) purified from bovine brains stimulate also the transcription and replication of VSV in vitro. The products of these reactions were intact mRNA or genome-sized RNA species. MAP stimulates the in vitro RNA synthesis by the VSV RNP--associated polymerase. Bovine brain MAPs added to influenza A in vitro transcription produced no stimulation (Hill *et al.*, 1986).

During the assembly of paramyxoviruses, nucleocapsids become aligned beneath areas of the plasma membrane rich of viral envelope proteins (Choppin and Compans, 1975). Portner and Kingsbury (1976) found the presence of M protein limiting the rate of Sendai virus essembly and budding. Giuffre *et al.* (1982) presented evidence for interaction of the membrane (M) protein of Newcastle disease and Sendai viruses with cellular actin. These results suggest that frequent identification of cellular actin in a number of enveloped viruses may be attributed to the interaction of actin and M protein or its equivalent. However, Griffin and Compans (1979) have presented evidence that the cytoskeleton is not involved in the maturation of vesicular stomatitis or influenza viruses. Genty and Bussereau (1980) also concluded that the cytoskeleton is not required for VSV production, but they did suggest that newly synthesized viral proteins, upon insertion into the host cell plasma membrane, induce a depolymerization of actin filaments.

Cellular morphology is generally altered after acute virus infection. The paramyxoviruses usually cause giant cell or syncytium formation as a consequence of their cytopathology. The agents (cytochalasin B, carbobenzoxy tripeptide SV-4814) which inhibit the ability of Vero cells to fuse after measles virus infection may be inhibitory to virus production. Actin network is essential to fusion (Bedows *et al.*, 1983). Cytochalasin D accelerates the release of Newcastle disease virus from infected cells (Morrison and McGinnes, 1985). The budding of measles virus from infected cells is inhibited after addition of anticalmodulin drugs trifluoroperazine and chlorpromazine (Bohn *et al.*, 1983). Chlorpromazine (CPZ) which binds specifically to calmodulin inhibits the activity of calmodulin stimulated 3', 5'-cAMP phosphodiesterase.

When CPZ is administered together with virus to chick embryo cells, CPZ reduces virus yields by 2-3 log PFU. Addition of CPZ 15 min before or 1 hr after influenza virus adsorption had no effect. The inhibitory action of CPZ was reverted by purified calmodulin (Križanová *et al.*, 1982). Cytochalasin B was reported to affect the spreading of the measles HA glycoprotein to the surface of persistently infected cells (Ehrnst and Sundquist, 1975, 1976). These results suggest that a functional cytoskeleton may play some role in the formation of infection virions. Electron microscopy using platinum- and carbon-shadowed cytoskeletons demonstrated that all stages of measles virus budding are associated with actin filaments composing the outer part of the cytoskeleton network; possibly the budding itself is the result of vectorial growth of actin filaments (Bohn *et al.*, 1986).

The transcription complex, the nucleocapsid with the viral RNA-synthesizing activity of NDV contains three protein components, the major structural subunit (NP) and two associated proteins (P and L) involved in the RNA synthesis. Most molecules of NP and P become associated with the cytoskeletal framework immediately after their synthesis. The cytoskeletal framework could actively participate in the structural and functional assembly of NDV transcription complex (Hamaguchi *et al.*, 1985). Bonneau *et al.* (1985) found that all mRNAs appear to associate with cytoskeletal structure during protein synthesis, irrespective of their 5′ and 3′ terminal structures. Cellular actin and viral (VSV and reovirus) mRNAs were released from the cytoskeletal framework and their translation was inhibited when cells were infected with poliovirus. The association of mRNA with the cytoskeletal framework is required but it is not sufficient for protein synthesis in eukaryotes.

The idea that cellular cytoskeleton is involved at some stages in replication of enveloped RNA viruses is a compelling one and has resulted in numerous studies over the years. The results presented here are fragmentary and far from comprehensive knowledge.

Adenoviruses

Many observations have suggested that microtubules and microfilaments or intermediate filaments are involved in the transport of material within cells and in the replicative cycle of adenoviruses.

Human adenovirus binds in vitro to reconstituted brain microtubules isolated from rodent cells (Lufting and Weihing, 1975) or from HeLa cells (Babiss *et al.*, 1979). The specific binding of adenovirus particles to microtubules has been termed "edgebinding", and has been shown to be mediated via microtubule-associated sidearm proteins (Weatherbee *et al.*, 1977). Dales and Chardonnet (1973), Miles *et al.* (1980) and Lenk and Penman (1979) demonstrated a hypothetical model of adenovirus infection which predicts a vectorial transport of infecting virions to nucleus via interactions with cytoplasmic microtubules in vitro. Luftig and Weihing (1975) and Weatherbee *et al.* (1977) presented the very similar hypothetical model based on results in vitro. Belin and Boulanger (1985) used BHK 21 cells as host system for

human adenovirus type 2 in order to identify some of the cytoskeleton elements transiently associated with or in close vicinity to intracytoplasmic adenovirus, and possibly involved in the virion transport to nucleus. Core proteins V and VII appeared to bind vimentin and α-tubulin with the highest efficiency, hexon protein showing a relatively weak reactivity on gel towards cytoskeletal proteins. These results confirmed previous investigations into the role of tubulin in the adenovirus transport (Luftig, 1982; Weatherbee *et al.*, 1977).

Adenovirus causes a dramatic shut-off of host protein synthesis after infection of HeLa cells. The level of actin mRNAs remained relatively unchanged after viral infection, when assayed by in vivo translation and two-dimensional gel electrophoresis analysis of the proteins or hybridization of the total cytoplasmic RNAs to the human actin gene. The distribution of actin mRNAs in the polyribosomes and monoribosomes of the infected cells occupied by virus messages are untranslatable in a rabbit reticulocyte lysate. The inhibition of actin synthesis after adenovirus infection of HeLa cells could be explained by the virus-coded or virus-induced alteration of a factor(s) unfavourable for translation of actin mRNA. The alteration implies a selective modification of the translational machinery affecting cellular mRNA translation and/or virus-induced inactivation and shift to smaller polysomes of actin mRNA. These alterations do not affect the size of the actin mRNA but only its ability to serve in translation (Khalili and Weinmann, 1984).

The previous results of van Venrooij *et al.* (1981) also indicate that the association of mRNA with the cytoskeleton is somehow related to its activity in protein synthesis. The functional importance of the association of mRNA with the cytoskeleton framework is still not clear. It is tempting to speculate that binding of mRNA to the cytoskeleton framework may be essential for efficient translation and that the availability of binding sites may be limiting. In that case shut-off of host protein synthesis after infection with adenovirus may be explained by competition between the population of cellular mRNA and the rapid increasing amount of viral mRNA for available cytoskeleton binding sites.

The microtubules are involved in the cytopathic effect of adenoviruses on host cells. The inhibition of paracrystal formation was observed 9 hr before the appearance of adenovirus-induced cytopathic effect. Cycloheximide blocked both the inhibition of paracrystal formation and the induction of the late cytopathic effect. These results suggest that an early protein synthesized de novo by adenovirus was required for direct or indirect inhibition of the biological functions of early proteins specified by adenoviruses. One of the biological functions of an early protein(s) of adenovirus is to affect microtubules resulting in the inhibition of paracrystal formations and causing shrinkage and cell rounding (Ebina *et al.*, 1978).

The nuclear matrix has been implicated in several important cellular processes. Electron microscopic examination of nuclear matrices isolated from adenovirus infected Hep-2 cells clearly revealed that late in the lytic cycle the adenovirus capsids are intimately associated with nuclear matrix.

Most of the virus endoproteinase activity co-purified with the nuclear matrix; these data suggest that the enzyme may be released from fragile young virions or assembly intermediates, that the nuclear matrix is the site of adenovirus assembly and that mature virions may be released from the matrix by the viral endoproteinase (Khitoo *et al.*, 1986). Ben-Ze'ev *et al.* (1986) demonstrated the cleavage of the intermediate filament protein vimentin in dense cell cultures and the inhibition of this cleavage when the cells are actively proliferating or when transformed by adenovirus 5. Therefore it si possible that the cleavage of vimentin in certain dense cells might be one of the means by which the highly insoluble vimentin network is reorganized. Such alterations in the reorganization of the vimentin network might be necessary in cells causing undergoing morphological changes occuring the transition from a spares to a dense monolayer. Further study is needed to characterize the protease responsible for the cleavage of vimentin, its role, if any, in the regulation of vimentin organization in vivo and the mechanisms by which cell transformation with adenovirus 5 brings about the inhibition of this cleavage in the vimentin molecule.

Poxviruses

Poxviruses are the largest, most complex animal viruses. Electron microscopical analysis of pox virus-infected cells has shown that virus replication and assembly occur within localized regions of the cytoplasm termed "factories" (Morgan, 1976). One of the striking features of pox virus-infected cells is the expression of microvilli-like surface protrusions which contain a mature virus at their top (Stokes, 1976). Microvilli-like protrusions are clearly different from nornal microvilli because of a threefold larger diameter. Their induction requires assembled virus particles. The specialized microvilli-like structures contain a core of microfilaments with a virus particle at their top. Within these pox virus-induced microvilli only α-actin, filamin and fimbrin could be positively identified as associated with the array of actin filaments. Inhibition of virus assembly prevents the induction of the specialized microvilli, thus indicating that mature viruses, or a factor present only under such conditions, induce their formation (Hiller *et al.*, 1979). The microvilli--type structures containing virus particles at the top of a bundle of microfilaments are very similar to those found in Rous sarcoma and mammary tumour virus-infected cells. The immunofluorescence data obtained by Hiller *et al.* (1981) clearly revealed that vaccinia-induced microvilli are devoid of microtubules and 10 nm filaments, but do contain F-actin and a variety of F-actin binding proteins. Colcemid-treated vaccinia virus-infected cells neither change the virus distribution nor the expression of specialized microvilli. Thus, the intimate association of the progeny virus with the cytoskeleton during cytoplasmic dissemination seems to involve solely actin-containing structures and is independent on the cellular display of intermediate filaments and the presence of microtubules. Meyer *et al.* (1981) found that in the presence of puromycin, sufficient to block vaccinia virus protein synthesis, the microfilaments were significantly preserved after infection. However, the mecha-

nisms mediating disappearance of the actin filament bundles during vaccinia virus infection of host cells are unknown.

Herpesviruses

Numerous electron microscopic studies on herpesviruses, mostly performed by the thin section technique, have presented information on virus morphology during maturation and assembly (Morgan *et al.*, 1959; Nii *et al.*, 1968; Cook and Stevens, 1970; Nii, 1971; Nii and Yasuda, 1976; Morgan *et al.*, 1976; Rajčáni and Čiampor ,1978; Dupuy-Coin *et al.*, 1978; Atkinson *et al.*, 1978; Fong *et al.*, 1979; Cavallo *et al.*, 1981; Čiampor *et al.*, 1981; Čiampor and Szántó, 1982; Bibor-Hardy *et al.*, 1982; Krempien *et al.*, 1984; Bibor-Hardy *et al.*, 1985). Rapidly accumulating evidence shows that the nuclear matrix may be the nuclear equivalent to the cytoplasmic skeleton or the eukaryotic equivalent of the bacterial membrane supporting DNA synthesis. The nuclear matrix is defined as a structure remaining after low salt and high salt sequence DNAse treatment of nuclei. The interaction of herpes simplex virus (HSV) with eukaryotic cells can be productive, abortive, transforming, persistent or latent. Viral DNA replication and its encapsidation occurs in the nucleus. Previous work of Bibor-Hardy *et al.* (1982) with HSV type 1 demonstrated that during productive infection, several virus-induced proteins become the part of the nuclear matrix; many viral capsids, mostly empty, remained attached to the internal fibrogranular network. The presence of viral proteins in the nuclear matrix has been confirmed for HSV type 2 (Tsutsui *et al.*, 1983).

These findings raised the question of the role of the nuclear matrix during virogenesis. This structure may be the site of viral capsid formation and/or where DNA is synthesized and stored before encapsidation. During infection of BHK-21 cells with HSV-1 the immediate-early protein species were bound to the nuclear matrix and their association with this structure was stable. During the first few hours p.i., the pattern of virus-induced proteins attached to the nuclear matrix remained identical, indicating that early polypeptides are not associated with this cell fraction. From the late proteins, which are mainly structural, 60% were tightly bound to the nuclear matrix. This suggests that the nuclear matrix is involved in at least two different events, namely the regulation of virus replication and the assembly of viral capsids (Bibor-Hardy *et al.*, 1985).

Quinlan and Knipe (1983) have shown that two HSV proteins, the major capsid protein (VP 5) and the major DNA binding protein (ICP 8) are specifically localized in the nucleus of infected cells. The major proportion of these proteins is associated with the detergent-insoluble matrix or cytoskeleton framework of the infected cell since their final binding in the nucleus. The DNA binding protein remains associated with the nuclear skeleton at times when it is bound to viral DNA. Thus, viral DNA may be attached also to the nuclear framework. Inhibition of viral DNA replication with phosphonoacetate enhanced the association of ICP 8 with the cytoskeleton and increased the exchange rate between cytoplasmic and nuclear framoworks,

suggesting a functional relationship between these events. Inhibition o viral DNA replication resulted in decreased synthesis and transport of the capsid protein.

Further results have shown that major capsid protein (VP 5) accumulates on the nuclear matrix, while ICP 8 accumulates in the chromatin fraction that can be separated from the nuclear matrix by extraction with DNAse and salt. VP 5 is transported after a lag phase from the cytoplasmic framework to the nuclear matrix, while ICP 8 is transported faster to the chromatin fraction (Ben-Ze'ev *et al.*, 1983). Prior to viral DNA synthesis, ICP 8 was found at discrete prereplicative sites throughout the nucleus, where it exhibited a high salt-labile association with the nuclear matrix. During replication of viral DNA ICP 8 was randomly distributed in the replication compartments being bound to viral DNA. The prereplicative sites may serve as nuclear reservoir for ICP 8 unbound to replicating or progeny DNA. Furthermore, when viral DNA replication was interrupted, ICP 8 moved back to the prereplicative sites. These experiments argue for existence of different sites of nuclear maturation for ICP 8 (Quinlan *et al.*, 1984).

Is the nuclear matrix involved in HSV DNA synthesis? The viral gene expression is regulated at the nuclear matrix level; the regulatory proteins would first bind to the nuclear matrix and, once associated, they mediate attachment of the viral DNA to stimulate transcription.

In normal cells, the nuclear matrix is a structure involved in the synthesis of DNA and its transcription, but in cells infected with viruses no functional relationship has been identified so far. Among the proteins of the early group, many are implicated in viral DNA synthesis or have regulatory functions similar to ICP 4, allowing transcription of late mRNA and inhibiting trasncription of immediate-early mRNA. Since viral DNA is firmly bound to the nuclear matrix, no early protein seems attached to the nuclear matrix. DNA-binding proteins attached to the nuclear matrix must be examined (Simard *et al.*, 1986).

Fibronectin, a dimeric glycoprotein, secreted to the cell surface is the substrate of fibroblast-like cells. It is lost from the surface of HSV-infected cells during cell rounding. Decrease and disarrangement of fibronectin is similar during cell rounding and cell fusion. Loss of fibronectin is closely connected with the cytopathic effect (CPE) and could not be prevented by protease inhibitors. The immediate-early protein 175 kD is essential for induction of CPE and loss of fibronectin (Dienes *et al.*, 1985).

Two and a half hours after infection with a high dose of different strains of HSV-1 which induce rounding of cells, breakdown of actin containing microfilaments can be observed (Heeg *et al.*, 1981; Heeg *et al.*, 1986). Also "early functions" of human cytomegalovirus were found to induce a loss of microfilaments. Actin synthesis in infected cultures, on the other hand, appeared to be largely unchanged (Löse *et al.*, 1982). The HSV-induced signals for early breakdown and the biochemistry of microfilaments during cell rounding as well as the pathway of the loss of microfilaments in cytomegalovirus-infected cells are completely unknown.

Other RNA and DNA viruses

The studies of frog virus 3 (FV3)-infected cells have indicated that cytomatrix filaments and the microtrabecular lattice may interact with viral assembly sites and be required for their formation and maintenance (Murti and Goorha, 1983; Murti *et al.*, 1984). These virus induced loci lack tubulin, vimentin, actin, or myosin but are surrounded by intermediate filaments (Murti *et al.*, 1984). Further studies of Chen *et al.* (1986) showed that FV3 assembles in morphologically distinct assembly sites in the cytoplasm of infected cells. As the assembly sites form, the intermediate filaments aggregate, delimit the assembly sites, and remain so throughout the infection. Vimentin is more acidic in FV3 infected cells than in uninfected cells. The acidification of vimentin was possible due to a fourfold increase in phosphorylation and the phosphorylation of vimentin preceded the reorganization of IF around virus assembly sites. The changes in IF organization are probably required for FV3 assembly site formation.

In contrast, immunofluorescence studies of Tipula iridescent virus (TIV)--infected Estigmene acrea cells have shown that cytomatrix filaments are not directly involved in formation or maintenance of TIV viroplasmic centres (Seagull *et al.*, 1985). Intact viroplasmic centres and viral assembly sites were isolated from E. acrea cells infected with TIV using monoclonal antibodies raised against lymphocyte nuclear proteins. This isolation indicated a possible involvement of highly conserved nuclear proteins in the assembly and maturation virions, as well as in maintaining the intergity of membrane-free viroplasmic centres. Electron microscopy and immunofluorescence of intact and fractionated E. acrea cells showed no evidence of cytoskeleton involvement in the formation and maintenance of TIV viroplasmic centres (Bladon *et al.*, 1986).

Infection of tissue culture cells with canine distemper virus caused a total reorganization of all cytoskeletal structures with the most notable changes in the microtubules and vimentin but not in keratin filaments. These structures may have a close association to canine distemper virus-infected cells (Howard *et al.*, 1983).

One of the first virus systems examined using selective extraction for biochemical and morphological studies were HeLa cells infected with poliovirus (Lenk and Penman, 1979). Viral RNA, specifically labelled in actinomycin-treated cells, was initially found completely attached to the skeletal framework both when replicating and when functioning as mRNA in polyribosomes. The release of viral RNA from the cytoskeleton occurs close to or coincident with virus maturation. The release of 35S genomic RNA from the skeleton is blocked in the presence of guanidine, which prevents the release of RNA from the replication site (Caliguiri and Tamm, 1968; Huang and Baltimore, 1970).

Aphthovirus, the foot and mouth disease virus (FMDV), a member of the Picornaviridae family, also revealed in replicative cycle a close cytoskeletal association of virus coded polypeptide derived from the P3ABC region of the viral polyprotein. The results of Grigera and Sagedahl (1986) support the

hypothesis of a close intracellular interaction of a short-lived polypeptide, containing the viral protease and VPg (capsid protein) sequence, with the host cytoskeleton, during infection of BHK cells with FMDV.

Further finding of Fraser and Whenham (1978*a*, *b*) that methylbenzimidazol-2-yl carbamate (MBC) inhibits the multiplication of tobacco mosaic virus (TMV) in tobacco leaves initiated the study of MBC effect on the assembly of TMV coat protein. The anti-microtubule agent MBC did not affect assembly of coat protein in vitro. In contrast, the anti-microtubule agents colchicine and vinblastine inhibited disk formation, but stimulated rod elongation from coat protein subunits in vitro. Both agents also disrupted preformed disks. Vinblastine inhibited virus multiplication in leaf tissue, but colchicine did not (Fraser *et al.*, 1986).

Reoviruses utilize the microtubules during the later phases of multiplication and assembly. Combined electron microscopic and autoradiographic studies indicate that the movement of ^{3}H-uridine-labelled genome RNA, presumably while still associated with the virus polymerase and other proteins of the core, can be traced to the vicinity of the microtubules. It is, therefore, conceivable that transcription and replication of the genome commences on or near to the microtubules (Dales, 1975).

Actin and simian virus 40 (SV40) viral structural polypeptide Vpl were found associated with fibres and fibre-associated electron-dense materials. Immunofluorescence as well as immunoelectron microscopic data suggest that fibres are intermediate filaments rather than microfilaments. The electron-dense material, with which actin and Vpl were specially associated in cytoskeletal preparations may be the remnants of subcellular structures which contain both actin and Vpl in intact cells. These results initiated the study of the synthesis of proteins and their transport to the interphase cell nucleus using SV40 as a model system. The mechanisms by which the transport of macromolecules from or to (or both) an interphase nucleus occurs is at present unknown (Kasamatsu *et al.*, 1983). Katsumoto *et al.* (1984) compared cytoskeletons of SV40-transformed cells with revertant cells or with normal cells. In all three cell types, microtubules were distributed similarly in the cytoplasm. But microfilaments in transformed cells were morphologically different from revertant or normal cells, i.e. decrease of the number and the length of microfilaments of transformed cells. It is possible to postulate, according to chromosome analysis, that gene dose affects the state of microfilaments in the cell. The revertant and normal cells had chromosome numbers of 94 (76-115) and 101 (86-107), respectively. The situation of microfilaments may affect the saturation density of cells.

Retroviruses and virus-transformed cells

Cultured murine epithelial cells, transformed with Moloney mouse sarcoma virus (MSV) or murine leukemia virus (MuLV), undergo different changes in both actomyosin and intermediate filament systems. Upon viral transformation, fibroblast cells lose the fibrillar actin organization rapidly and this may account for the major morphological changes in transformation. The

major change after transformation is the decrease of the actomyosin containing belt extending from cell to cell at the borders of the cell islands. The virus-transformed cell lines showed enhanced vimentin-specific fluorescence and similar molecular forms of keratin polypeptides were seen in all cells by immunoblotting. Viral transformation of epithelial cells thus leads to different changes in their cytoskeletal organization depending on the transforming viral or cellular gene (Keski-Oja *et al.*, 1983).

The studies of Edbauer and Naso (1983) have shown a rapid and specific association of Rauscher murine leukemia virus (R-MuLV) core precursor polyprotein $Pr65^{gag}$ with cytoskeletal elements in infected mouse fibroblasts. The cytoplasmic face of the cell membrane is clearly implicated for the critical region of retrovirus assembly (Yeger *et al.*, 1978; Demsey *et al.*, 1979). Numerous studies indicate that $Pr65^{gag}$ is the viral component necessary for retrovirus assembly and budding (Naso *et al.*, 1982). Furthermore, the post--translational association of $Pr65^{gag}$ with membranes has been strongly indicated as being essential for virus assembly (Yeger *et al.*, 1978; Bolognesi *et al.*, 1978). The cytoplasmatic face of the plasma membrane is the region of the cell where integral membrane proteins interact with cytoplasmic proteins and with components of the cytoskeletal system (Nicolson, 1976; Simons and Garoff, 1980). Based upon the known function of R-MuLV precursor polyprotein $Pr65^{gag}$ in virus assembly, the specificity of its association with the cytoplasmic face of the cell membrane, the stability of cytoskeletal $Pr65^{gag}$, and its presence in virus particles in uncleaved form, Edbauer and Naso (1983) proposed a model for the active role of cytoskeleton associated $Pr65^{gag}$ in virus assembly. The region of the $Pr65^{gag}$ molecules involved in the association with the cytoskeletal elements is unknown. The fact that viral p30 appears to be the only mature viral core protein found in the skeletal fraction suggests that it is the p30 region of $Pr65^{gag}$ that is involved in the association. Edbauer and Naso (1983) postulate that $Pr65^{gag}$, anchored to cytoskeletal elements, functions not as a significant source of viral structural proteins, but rather as a stabilizing element in the virus assembly site and in newly budded virions. It is also probable that skeleton-anchored $Pr65^{gag}$ into assembly sites in the infected cell. The association of functions to direct and otherwise regulate the lateral clustering of unanchored, membrane-associated $Pr65^{gag}$ with cytoskeletal elements may also explain the presence of cytoskeletal proteins in mature virus particles.

The role of microtubules in the replicative cycle of the enveloped retroviruses is not yet clear and may vary from one virus system to the other. In vitro, Moloney murine leukemia virus production is severely inhibited when microtubule-depolymerizing drugs are present in cell medium (Satake and Luftig, 1982). Likewise, association of the p27 Rous sarcoma virus internal protein with microtubules has been suggested (Stanislawski, 1983*a*). During the early period of Rous sarcoma virus release from infected chick embryo fibroblasts, virions in close association with the extracellular fibrillar matrix were observed (Stanislawski, 1983*b*). However, the nature of linkage between microtubules and virus particles is still uncertain. The findings of Pepper and

Brinkley (1977) show the presence of tubulin on the surface of A type particles in CHO cells.

Vincristine sulphate inhibits both microtubule polymerization and virus release, suggesting that intact cytoplasmic microtubules are necessary for intracellular transport and release of virus. Immunoelectron microscopic studies showed that neither tubulin nor actin could be identified on the surface of A type particles (Heine *et al.*, 1985).

Transformation of chick embryo fibroblasts in vitro by Rous sarcoma virus brings about a variety of phenotypic changes including rounding of cells and alterations in their adhesiveness (Weiss *et al.*, 1983). Cell transformation is mediated by the protein product of a single transforming gene called *src* (Wang *et al.*, 1975). Actin and tubulin which are normally depolymerized in RSV-transformed cells appear unaffected by avian sarcoma virus UR2 transformation. Cell surface fibronectin which is normally lost from RSV--infected cells, seems more abundant on UR2-transformed cells than on normal cells. The cell associated fibronectin synthesized during the labelling period is reduced by 60% in RSV-transformed cells but occurs in normal amounts in UR2-transformed cells. These data suggest that more of the fibronectin binds to the surface of UR2-transformed cells than to normal cells, but is readily released from RSV-transformed cells. Vinculin was reduced by about 50% of normal levels in both RSV- and UR2-transformed cells. This indicates a cytoplasmic location or membrane association for $p68^{ros}$, the transforming protein of UR2, which contains *gag* determinants. Overall, these data suggest that changes in the major cytoskeletal proteins of fibroblasts are not essential for the neoplastic properties of cells but rather a phenotypic expression of transformation, since UR2, which causes tumours in vivo, induces only minor cytoskeletal alterations of cells transformed in vitro (Notter and Balduzzi, 1984).

The *src* phosphoprotein has been shown to phosphorylate vinculin (Sefton *et al.*, 1981) a cytoskeletal protein found in focal adhesion plaques (Geiger, 1979). Disorganized microfilaments (Wang and Goldberg, 1976) and altered microfilaments and microtubules (Edelman and Yahara, 1976; McClain *et al.*, 1977) were observed following transformation by RSV as well. Moreover, early membrane alterations and "flower" formation indicate a direct involvement of pp^{src} in the cellular cytoskeleton (Boschek *et al.*, 1981; Boschek, 1982; Shriver and Rohrschneider, 1981).

Numerous studies indicate that tumour promotor induces rapid and coordinate reorganization of actin and vinculin in cultured cells (Schliwa *et al.*, 1984). Fibronectin becomes linked to tumour promotion (Zerlauth and Wolf, 1985). Tubulin and actin synthesis decreases in 3T3 cells after transformation by SV40 virus (Fine and Tayler, 1976). Cytoskeleton changes and expression of the H-ras oncogene occur during promotion of neoplastic transformation in mouse epidermal JB6 cells (Takahashi *et al.*, 1986). The role of tubulin and actin in paired nonneoplastic and spontaneously transformed neoplastic cell lines in vitro has been studied recently (Tucker *et al.*, 1978).

The ultrastructure of mammary epithelial cells and Rous sarcoma virus--transformed chick embryo fibroblasts shows that virus extrusion is associa-

ted with filament-containing cellular processes. In particular, MuMTV is released from the ends of long microvilli which contain a bundle of 6—8 nm microfilaments and share some structural features with intestinal microvilli. Damsky *et al.* (1977) suggest that virus nucleoids require interaction with host cell contractile proteins for their extrusion from cell.

Protein kinases are thought to play a key role in signal transduction and oncogenesis, but little is known about the intranuclear phosphorylation events associated with transformation. Cellular 350 kD protein is an immunological analogue of high molecular weight microtubule-associated protein 1 (MAP-1) of brain. Persistent intranuclear location of the phosphorylated 350 kD protein was also found throughout the cell cycle in transformed cells. The 350 kD protein seems to be a target molecule of protein kinases that are stimulated directly or indirectly by growth factors or by oncogene products in the nucleus, and appear to represent a new transformation-related nuclear antigen (Sato *et al.*, 1986).

Conclusions

The cytoarchitecture concept emphasizes the fibrous, polymeric configurations of cytoplasmic proteins, such as microfilaments, intermediate filaments, and microtubules. Cytoplasm is a giant multienzyme complex (Albrecht-Buehler, 1985) and in view of this pattern, new mechanisms of control of chemical and molecular biological reactions become conceivable. With these spatial arrangements of molecules, it becomes likely that there is a cooperation between biochemical events over large intracellular distances.

In light of the cytoarchitecture concept I have attempted in this review to cover what is known about how cytoskeletal and nucleoskeletal elements are involved in replicative cycle of viruses and transformation of cells. The association of virus metabolism with cell architecture is a new area and much work remains to be done till our understanding of all these associations, functions and processes.

The recent novel results indicate that microtubule depolymerization by colchicine and other drugs is sufficient to initiate DNA synthesis in serum-free cultures of embryonic fibroblasts and that stabilization of microtubules with taxol inhibits this initiation. Growth factors and oncogenic DNA viruses also initiate DNA synthesis by a taxol-sensitive mechanisms that appear to require microtubule depolymerization or rearrangements. The slight changes in the extent of microtubule assembly or changes in specific functional groups of microtubules may initiate intracellular signals leading to cell proliferation (Carney *et al.*, 1986).

Since viral replication has indicated a clear involvement of the cytoskeleton or nuclear matrix in virus metabolism, further biochemical, molecular biological and morphological studies are needed in order to establish more precise correlations between the numerous ultrastructural lesions described in virus-infected, virus-transformed and cancer cells. In addition, the avenues are open for further investigation.

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