Adenovirus core protein V is delivered by the invading virus to the nucleus of the infected cell and later in infection is associated with nucleoli

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Virus capsid proteins play a key role in the recognition of cell receptors and consequent adsorption and penetration of the virus. In the case of adenoviruses there is a complex capsid structure comprising seven different proteins. The major capsomere is the hexon of which there are 240, forming an icosahedral shell with the 12 apices of the icosahedron accommodating pentons which are themselves bipartite, consisting of the penton base and fibre. A number of other proteins, viz. VI, VIII, IIIa and IX, interact to stabilize this structure and to contact the nucleoprotein core of the virus. The latter consists of a linear double-stranded DNA genome, with a covalently linked terminal protein (TP) at each 5′ terminus, in close association with the highly basic protein VII and a more loosely associated protein V. The locations of the major components of the capsid have been well-defined and tentative assignments have been made for the other capsid proteins based on cryoelectron microscopy and knowledge of the structure of the hexon from X-ray analysis (Stewart et al., 1993). The disposition of the core proteins is not so well-established but there have been indications that the genome is held in a tightly coiled configuration. A number of these structural proteins are subject to specific processing by a virus-coded protease which also appears to be incorporated within the mature capsid (for a review see Weber, 1995).

The initial interaction of the virus with the host cell occurs via the knob of the fibre and components of the cell receptor have recently been defined (Bergelson et al., 1997; Hong et al., 1997; Tarassishin & Russell, 1997). The penton base also fulfils a role in these early events by interacting with cellular integrins to promote fusion with endosomal membranes and penetration of the virus via the endocytic pathway has been well-established, although other factors such as the integrity of the capsid seem to be important (Wickham et al., 1993; Russell & Kemp, 1995).

There have been a number of studies which suggest that the infecting virus gradually disassembles on entry and penetration into the host cell (Greber et al., 1993, 1996). The drop in pH which occurs in the endocytic pathway may also have a part in destabilizing the capsid structure, especially in modulating the tight association between hexons and protein VI. This latter protein appears to be critical in retaining the integrity of the capsid structure, being in a key position proximal to the so-called peri-pentalon hexons. The validity of this association has been verified by our studies on protein–protein interactions between hexon and polypeptide VI (Matthews & Russell, 1994, 1995). We demonstrated an association between these two proteins which was enhanced very significantly after processing of the pre-polypeptide VI by the virus-coded protease, suggesting that some conformational change takes place on maturation. Thus a destabilized capsid would be delivered by the endocytic pathway to the cytoplasm and since the adenovirus genome functions in the nucleus some mechanisms for further disassembly of the virion and for delivery of the virus genome must be called into play.

In an attempt to throw some light on these mechanisms we reasoned that protein V, which appears to be associated with both the core and capsid components of the virus particle, might play a role. We expressed protein V by cloning the PCR-amplified gene into pRSETA (Invitrogen) via BamHI sites, using techniques previously described (Matthews & Russell, 1994). This procedure placed the gene downstream of a T7
Fig. 1. Blotting of purified viruses and infected cells using rabbit V antiserum. Duplicate blots were made after SDS–PAGE of the following samples. Lane 1, purified wt Ad2 virus; lane 2, purified ts1 Ad2 virus (grown at the restrictive temperature); lane 3, extracts of Ad2 infected cells; lane 4, extracts of uninfected HeLa cells. (a) Stained with Naphthalene black. Molecular mass standards on the left (M) and the relative mobilities of the relevant adenovirus structural proteins on the right. (b) Western blot with rabbit polyclonal anti-V serum. (c) ‘West-Western’ blot using a ‘wash’ of rV at 10 ng/ml and then detection of bound rV with the V antiserum.

RNA polymerase promoter and inserted a ‘six histidine’ at the N terminus of the expressed recombinant V (rV) polypeptide. Purification of rV under denaturing conditions (viz. 6 M guanidine.HCl, 20 mM mercaptoethanol) using a ‘nickel column’ gave a product with the expected electrophoretic mobility on SDS–PAGE although smaller polypeptides were also evident, presumably as a result of proteolysis. Nevertheless, by intramuscular immunization of this material along with Freund’s complete adjuvant (Sigma; 1:1, final volume 300 µl) followed by appropriate boosting, an antiserum was obtained which clearly detected polypeptide V by Western blotting in purified wt and ts1 virus preparations (Fig. 1b). In the latter case, the protease is not functional and the non-infective virus contains unprocessed structural proteins.

Methods for gel electrophoresis and Western blotting and preparation of purified virus and cell extracts have been described previously (Matthews & Russell, 1994). In both virus preparations there was also a minor band of slightly slower electrophoretic mobility (Vb). Extracts of infected cells gave a similar pattern but in addition a major antigenic product of 39 kDa was also evident (Fig. 1b, lane 3). There was no reaction with uninfected cells. We carried out ‘pulse–chase’ experiments to determine if the larger polypeptide (Vb) was the result of processing but were not able to demonstrate such an event. It is relevant to note that a similar observation of Vb was made (but not discussed) by Winter & d’Halluin (1991) using another antibody. In a series of experiments preparations of purified virus from both adenovirus type 2 (Ad2) wt and Ad2 ts1 and from extracts of wt infected and uninfected cells were analysed by SDS–PAGE and replicates of the separated polypeptides blotted onto nitrocellulose. Fig. 1(a) shows the polypeptide pattern of the blots as visualized by staining with Naphthalene black, by Western (Fig. 1b) and ‘west-Western’ (Fig. 1c) blotting with the rabbit V antiserum. In the latter procedure after appropriate washing of the blot it was incubated with buffer containing purified rV and finally the washed blot was probed with the rabbit V antiserum (for details of procedure see Matthews & Russell, 1994). Fig. 1(c) indicates that protein V bound to both polypeptides VI and VII and apparently to itself in the purified virus preparations (lanes 1 and 2). The result in the case of Ad2 ts1 suggests that V bound to polypeptide pVII but not to pVI. These findings are in keeping with the suggestion that protein V acts as a bridging protein between the nucleoprotein core and the capsid and is in agreement with a previous observation using a different method (Chatterjee et al., 1985). Moreover, the inability of protein V to bind to protein pVI under these conditions may be another indication that considerable conformational changes of pVI and VI occur on virion maturation by the virus protease (cf. Matthews & Russell, 1995). It is interesting that in the cell extracts (lanes 3 and 4) protein V binds to polypeptides of ~ 70 kDa and 16 kDa, suggesting that recognition of cellular components may be of some significance in virus–cell interactions. This theme is developed in another series of experiments (accompanying paper: Matthews & Russell, 1998).

In an effort to establish the location of polypeptide V in infected cells immunofluorescent double-labelling studies by confocal microscopy were carried out using the rabbit V antiserum (as well as a monoclonal antibody against V generously supplied by Jane Flint) in combination with antibodies against adenovirus structural and non-structural components. By infecting cells at a high multiplicity (and achieving some degree of parasynchrony by adding virus seed initially at 4°C) and then fixing with paraformaldehyde followed by a detergent-containing buffer, protein V could be readily detected in the cell cytoplasm at 30–60 min post-infection (p.i.), presumably reflecting antigen made available by
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Fig. 2. Confocal fluorescence micrographs of adenovirus-infected cells. Antigens were detected using antibodies as indicated below and detected with fluorescein-conjugated anti-mouse IgG and Texas red (or rhodamine)-conjugated anti-rabbit IgG. Cells were fixed with acetone–methanol (1:1) at —20 °C for 10 min or with 4 % paraformaldehyde in buffer A for 5 min at room temperature, followed by treatment with 0.5 % Triton X-100 in buffer A at 4 °C for 5 min (for further details see Matthews & Russell, 1998). Cells were infected at either a high multiplicity of about 100 p.f.u. per cell to examine early events or at about 1 p.f.u. per cell for late events. White bars, 10 µm. (a) Ad2-infected HeLa cells: 60 min p.i. with rabbit anti-hexon (red) and mouse monoclonal anti-V (green); fixed in acetone–methanol. (b) Ad2-infected HeLa cells: 60 min p.i. with rabbit anti-protein VI (red) and mouse monoclonal anti-V (green); fixed in acetone–methanol. (c) Ad2-infected HeLa cells: 15 h p.i. with rabbit anti-V (red) and mouse monoclonal anti-DNA-binding protein (green); fixed in paraformaldehyde–Triton. (d) Ad2-infected HeLa cells: 15 h p.i.; rabbit anti-V (red) and monoclonal anti-nucleoli (green); fixed in paraformaldehyde–Triton.

the infecting virus as well as that derived from defective virus particles (data not shown). By utilizing acetone–methanol at —20 °C better penetration of the nucleus was obtained and at 60–90 min some of the V antigen (green) could be detected at the nuclear membrane in association with hexon antigen (red), giving a yellow-orange fluorescence; however, a clear separation of V antigen from hexon as V moved into the nucleus could be discerned (Fig. 2a). A similar result showing separation of V from protein VI was obtained using an antibody to protein VI (Matthews & Russell, 1994, Fig. 2b). The nuclear location of V was confirmed by viewing sections through the nucleus. At later times (> 120 min) no V antigen could be detected in the nucleus although both cytoplasmic hexon and V antigen were seen. At even later times (> 4 h) the cytoplasmic V antigen diminished but the residual hexon antigen appeared to be retained (data not shown). The observation of the relatively rapid transport of protein V to the nuclear membrane is in keeping with earlier observations using electron microscopy by Dales & Chardonnet (1973) and the biochemical approaches by Greber (1993, 1996). Our findings suggest that the association between the core and capsid via protein VI is disrupted on virus entry and the virus core, viz. virus DNA and associated proteins, enter the nucleus quite rapidly. The accompanying loss of V antigenicity in the nucleus presumably reflects either poor accessibility of the antigen to the antibody in the nucleus or more likely the masking of the antigen as the template is anchored via TP to the nuclear matrix and early transcription gets under way. If this latter suggestion is accepted then it could be postulated that the early transcription patterns seen in adenovirus infection may result from selective template masking by core proteins. Furthermore, since the virus core on its own can act as a template for in vitro DNA replication (Leith et al., 1989) it is not unreasonable to suggest that such an event could also occur in vivo yielding progeny DNA molecules to act as templates for late transcription. It is notable that polypeptide V harbours a bipartite nuclear location signal (NLS) which is retained in all adenovirus V sequences published (see Russell &
Kemp, 1995) as well as other NLS motifs and these may play a role in directing the virus genome into the nucleus.

When virus late transcription and polypeptide synthesis get under way at about 12–16 h p.i. protein V could be detected in the nucleus as small aggregates proximal to the nuclear membrane and then in the form of large prominent nucleolar-like rings – in some cases only one large ring was observed. These rings were quite distinct from the early non-structural protein, the DNA-binding protein, which assembles in characteristic patterns as previously described (Hayashi & Russell, 1968) (see Fig. 2c, d). As infection proceeded the V antigen seemed to fill all the nucleus and then diminished, presumably reflecting masking of the antigen as the virus was encapsidated (data not shown). It is also notable that some of these stages could be observed in the same preparations, reflecting the heterogeneity resulting from the relatively low m.o.i. To assess the relationship to nucleoli, double-labelling was carried out using a monoclonal antibody specific for nucleoli (Chemicon) and the rabbit V antibody. Fig. 2(d) clearly shows that the V antigen associates initially with periphery of the nucleoli as exemplified by the patchy orange fluorescence. Thereafter the nucleoli appeared to stain uniformly orange but this could result from accumulation of V at their periphery rather than ingress. It is interesting that another adenovirus gene product, IVα2, has also been detected both in the nucleoplasm and in association with nucleoli (Lutz et al., 1996). This protein appears to play a role in the activation of the adenovirus major late promoter (thus controlling synthesis of V) and this property is consistent with its location in the nucleoplasm in the fibrogranular network where transcription is deemed to occur. It has been suggested by Lutz et al. (1996) that the nucleolar localization of IVα2 suggests that it carries out another function in virus infection. Perhaps the nucleolar localization of protein V indicates that it too may have some role in the adenovirus life-cycle other than that of a structural protein. Attempts have been made to characterize the nucleolar localization signals in both adenovirus IVα2 (Lutz et al., 1996) and in herpesvirus ICP27 [which has a similar distribution in the nucleus (Mears et al., 1995)] and it was noted that neither of them conformed to the consensus motifs previously established but appeared to harbour a number of NLSs sometimes extremely rich in arginine. These latter observations would certainly be in keeping with the sequence data on adenovirus protein V.

Using electron microscopy, other investigators (Rebelo et al., 1996; Lutz et al., 1996) have described a range of nuclear and nucleolar (Puvion-Dutilleul & Christensen, 1993) alterations following adenovirus infection. It has been postulated that these events are related to the complex splicing patterns which occur during infection. It is interesting to note that protein V is one of the cassette of proteins which is regulated by alternative splicing mechanisms (Kreivi & Akusjarvi, 1994) and although we have been unable to colocalize the patterns of nuclear V antigen with splicing factors using confocal microscopy and appropriate antibodies (data not shown) it should be instructive to examine the development of V protein by electron microscopy.

We are indebted to Jane Flint (Princeton) who generously supplied anti-V monoclonal antibodies.

We wish to thank John Mackie for assistance with confocal microscopy, Bill Blyth and Jim Allen for photography and Margaret Wilson and Margaret Smith for preparation of the manuscript.

D.A.M. was in receipt of MRC Studentship G78/1131.

References


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Received 12 February 1998; Accepted 27 March 1998