Adenovirus Signalling in Entry

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Adenovirus Signalling in Entry

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Abstract

Viruses carry nucleic acids between and within host cells. Invariably, virus attachment to host cells leads to activation of cell signalling. These so-called forward signals emerge from interactions with cell surface receptors or cytosolic proteins and elicit profound responses in the cells, for example induction of growth or innate immunity responses. They can enhance or suppress infection. In addition, viruses receive signals from the cell. These reverse signals can impact on the structure of the virus leading to genome uncoating. They can enhance infection or inactivate virus, for example by facilitating degradation. Here we discuss the nature and mechanisms by which forward and reverse signals emerge and affect the outcome of human adenovirus infections. We describe how human adenoviruses use cell surface receptors for forward signalling to activate cell growth, intracellular transport or innate immune response. We also discuss how adenoviruses use acto-myosin, integrins or microtubule-based kinesin motors for reverse signalling to facilitate their stepwise uncoating program.

Introduction

Viruses are diverse in nature and appearance. They range from small non-enveloped single stranded RNA or DNA viruses to lipid bearing double stranded RNA or DNA viruses with haploid or diploid genomes. Despite large diversity, they share common principles enabling infection. One invariable feature is that they protect their genome with a proteinaceous capsid and sometimes a lipid envelope. Capsid or envelope proteins dock to cellular receptors or soluble proteins. Another conserved feature between viruses is that they activate cell-signalling processes as soon as they interact with cells. In some cases this leads to the stimulation of endocytic uptake processes, such as clathrin-mediated endocytosis or macropinocytosis (Gastaldelli *et al.*, 2008, Amstutz *et al.*, 2008, Mercer *et al.*, 2009), the interference with the cell cycle or induction of cancer (Damania, 2007). Many aspects of adenovirus (AdV) biology have been intensively studied, and rapid progress occurs in areas of virus structure and entry, replication, pathology and immunology, evolution, vector development and vaccinations (recently reviewed in Greber *et al.*, 2012). Here we discuss how human adenoviruses (HAdV) induce signalling to the cell during entry, and how the viruses receive signals from the cell by a reverse process.

Forward signalling from cell surface receptors

Cell surface receptors mediate initial contacts of viruses to cells, and this can trigger cell signalling. If virus-receptor interactions are of high affinity, a few hundred receptor molecules per cell are sufficient for infection. If the interactions are of low affinity (in the micro-molar or even milli-molar range), or if the binding site on the receptor is masked, increased numbers of receptors are required for virus attachment. This can occur through avidity binding. Viruses are able to use avidity binding since they often display multiple receptor binding sites in close spatial proximity. In the case of HAdVs, this has been demonstrated for binding of HAdV-3/7 to the membrane cofactor CD46 (Trinh *et al.*, 2012). Furthermore, C-species HAdV

(HAdV-C) also bind with a large spectrum of affinities to their receptor, the coxsackievirus adenovirus receptor (CAR) (Kirby *et al.*, 2001). Low affinity, high avidity binding allows viruses to use multiple receptors depending on receptor availability, and this may broaden virus tropism. In the following section, we discuss the current picture of attachment factors, entry receptors, and supporting bridging factors for HAdV infections (for an overview, see Fig. 1).

The coxsackievirus adenovirus receptor CAR

AdV were first isolated in the 1950s (Rowe *et al.*, 1953). However, the identification of the first AdV receptor (CAR) took more than 40 years (Bergelson *et al.*, 1997, Tomko *et al.*, 1997). CAR is involved in the formation of tight and adherens junctions between epithelial cells (Cohen *et al.*, 2001, Walters *et al.*, 2002). It belongs to the immunoglobulin superfamily and is expressed in heart, brain, pancreas, intestine and at low levels in liver and lung (Fechner *et al.*, 1999). CAR interacts with the fiber knobs from HAdV of all species, except those from species HAdV-B (Roelvink *et al.*, 1998). Interestingly, the HAdV fibers bind to the same extracellular domain of CAR (the D1 domain), which is also engaged in homophilic CAR-CAR contacts between neighbouring cells, however, with much higher affinity (Bewley *et al.*, 1999, Freimuth *et al.*, 1999). In contrast to CAR dimerization, the attachment of viruses to CAR has been reported to elicit intracellular signalling that leads to an inflammatory response and expression of cytokines in human respiratory cells (Tamanini *et al.*, 2006). In addition, CAR overexpression studies in epithelial cells suggested that CAR is directly or indirectly involved in the activation of p42/44 extracellular receptor kinases (ERK) and could enhance $\beta_{1/3}$ integrin expression (Farmer *et al.*, 2009). The cytoplasmic domain of CAR appears to be involved in ERK activation (Farmer *et al.*, 2009), but can be dispensable for HAdV cell entry (Wang *et al.*, 1999, Burckhardt *et al.*, 2011). This is consistent with chemical interference studies showing that ERK signalling is not required for virus entry but rather plays a critical role in the inflammatory process

triggered by the virus (Bruder *et al.*, 1997, Suomalainen *et al.*, 2001, Smith *et al.*, 2011).

The membrane cofactor CD46

How HAdV-B species bind to blood and epithelial cells has remained unknown until recently. In 2003/04, four groups reported that the membrane cofactor CD46 was required for binding of HAdV-3, 11, 16, 21, 35, 50 (species HAdV-B1 and B2), as well as HAdV-37 (species HAdV-D) to epithelial and hematopoietic cells (Gaggar *et al.*, 2003, Segerman *et al.*, 2003, Sirena *et al.*, 2004, Wu *et al.*, 2004). More recently, HAdV-26 and 49 (species HAdV-D) were also found to use CD46 as a receptor (Lemckert *et al.*, 2006, Li *et al.*, 2012). These studies showed that all HAdV-B attach to cells via their fiber knobs, which recognize the extracellular domain of CD46, albeit with different affinities (Pache *et al.*, 2008, Cupelli *et al.*, 2010). This was concluded from a variety of biochemical and cell biological experiments and infection assays, including cDNA expression in receptor-negative non-human cells, virus-receptor co-localizations at the ultra-structural level, in-solution binding assays, mass spectrometry, antibody inhibitions of cell binding and infection, competition experiments with soluble fiber or receptor domains, or RNA interference in cultured or primary cells. CD46 is expressed on all nucleated cells in humans, and shields autologous cells from complement attack (for a review, see Riley-Vargas *et al.*, 2004).

Interestingly, ligand-induced CD46-oligomerization triggers macropinocytosis and down-regulation of CD46 (Crimeen-Irwin *et al.*, 2003), which in turn increases immune suppression and complement-mediated lysis. HAdV-3/35 (HAdV-B species) use CD46 for infection, and they enter and infect epithelial and hematopoietic cells by triggering macropinocytosis (for an overview of the pathway, see Fig. 2, and Amstutz *et al.*, 2008, Kalin *et al.*, 2010). These viruses also use the C-terminal binding protein 1 of E1A (CtBP1), which is a phosphorylation target of p21-activated kinase (PAK) 1.

CtBP1 is a multifunctional protein involved in transcriptional repression and membrane trafficking, including PAK1-mediated macropinocytosis (Liberali *et al.*, 2008). HAdV-3 entry activates PAK1 through Rac1, and both PAK1 and Rac1 are required for HAdV-3 or 35 infections (Amstutz *et al.*, 2008, Kalin *et al.*, 2010). Macropinosomes positive for fluorescent fluid phase marker dextran contain viruses and CD46, α_v integrin, CtBP1 and PAK1. Collectively, the data show that HAdV-B bind to CD46 and induce an endocytic pathway, which is used in antigen presentation and cell migration in non-polarized cells. The HAdV-B entry pathway may modulate immune responses possibly by interference with the transcriptional co-repressor CtBP1. Whether CD46 has a receptor function for HAdV-B on polarized cells lining tissue epithelia has not been reported so far.

The cell adhesion molecule desmoglein 2

Based on cross-competition data between HAdV-B types in different cells and neutralizations of infections with fiber knobs, it was widely assumed that there are additional attachment receptors for HAdV-B species, besides CD46. Using blot overlay techniques on SDS-denatured gel fractionated extracts from receptor positive and negative cells in combination with mass spectrometry, desmoglein-2 (DSG-2) was identified as an attachment receptor for HAdV-3, 7, 11, 14 (Wang *et al.*, 2011). DSG-2 belongs to the cadherin family of calcium-binding trans-membrane glycoproteins and is a component of the adhesion complexes between epithelial cells. The affinities of fibers to DSG-2 are weak, as the trimeric fiber knob from HAdV-3 did not bind to DSG-2, unlike intact HAdV-3 or penton-fiber complexes, so called dodecahedrons. This was similar to CD46, which bound to HAdV-3 or dodecahedrons by an avidity mechanism (Sirena *et al.*, 2004, Trinh *et al.*, 2012).

Intriguingly, the binding of AdV or dodecadehrons to DSG-2 elicited events similar to epithelial-to-mesenchymal transition (EMT), as evidenced by marker profiling and

p42/44-ERK or phosphatidyl-inositol-3 kinase phosphorylations (Wang *et al.*, 2011). Virus-induced EMT apparently opened up intercellular junctions. In the context of an epithelium, this could lead to the exposure of basolateral receptors towards the luminal side, for example the airway lumen, and thereby enhance the accessibility of the epithelium to exogenous agents and enhance infection. This is reminiscent of earlier reports showing that the release of excess fiber proteins from AdV infected cells disrupted cell-cell contacts and promoted the release of newly synthesized viruses from epithelia (Walters *et al.*, 2002).

Sialic acid and bridging factors

Highly abundant structures in glycoproteins or glycolipids of the cell surface are the negatively charged sialic acids. HAdV-8, 19, 37 (species HAdV-D) contain positive charges in their fiber knobs and bind sialic acid (Nilsson *et al.*, 2011). These interactions, however, do not account for the ocular tropism of species HAdV-D, since sialic acid is not specific for cell of the conjunctiva. Possibly, virus interactions with sialic acid contribute to AdV hemagglutinations, given that the erythrocyte surface is rich in the sialic acid bearing glycophorin A. It is also possible that virus attachment to sialic acid enhances the binding to other low affinity attachment sites, from which signals are transduced to the host cell.

Other low affinity attachment sites for AdV are heparan sulfate containing proteoglycans (HSPG) of the extracellular matrix. They comprise trans-membrane syndecans and glycosyl-phosphoinositide-linked glypicans. HSPG were reported to bind the fiber shaft of HAdV-C through a lysine-lysine-threonine-lysine (KKTK) motive (Dechecchi *et al.*, 2001, Darr *et al.*, 2009). Recently, HSPG was suggested as an attachment site for mouse AdV-1 on endothelial cells (Lenaerts *et al.*, 2012), and for assisting HAdV-3 attachment to integrin (Tuve *et al.*, 2008, Gout *et al.*, 2010). The precise role of HSPG in AdV infection remains elusive, however, also because the

affinity of AdV to HSPG is apparently much lower than of Herpes virus type 1 (Shukla *et al.*, 1999, Kalin *et al.*, 2010). Accordingly, little information exists for potential signalling events from bridging factors in HAdV entry. For further information on coagulation factor X, lung surfactant di-palmitoyl-phosphatidylcholine or lactoferrin, the reader is referred to Fig. 1, and a review (Arnberg, 2009).

Integrins as signalling receptors

Integrins are adhesion receptors, consisting of an α and β subunit, and connect cells to the extracellular matrix (ECM). They transduce outside-in or inside-out signals, and regulate cell survival and migration. Integrins were discovered as an AdV receptor long before CAR was known. An initial key observation was that soluble penton base from HAdV-2 precluded the attachment of α_v integrin-positive cells to culture dishes (Wickham *et al.*, 1993). Soluble penton base did not interfere with virus attachment to cells but inhibited virus endocytosis, similar to soluble RGD (arginine-glycine-aspartate) peptides, a motif also present in penton base. The RGD motive is found in all HAdV penton bases, except HAdV-40/41 (species HAdV-F), allowing these viruses to use $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_3\beta_1$ or $\alpha_M\beta_2$ integrins depending on the cell type (reviewed in Stewart *et al.*, 2007).

The best-characterized integrins in HAdV entry are $\alpha_v\beta_3$ and $\alpha_v\beta_5$ expressed on many epithelial cells (for a schematic drawing, see Fig. 3A). Both of them promote virus internalization, depending on the penton base RGD or an engineered RGD in the fiber knob (Wickham *et al.*, 1993, Nagel *et al.*, 2003). This suggests that HAdV use integrins that are in an activated state, and transduce signals, for example to phosphatidylinositol-3-OH kinase (PI3K) (Li *et al.*, 1998b). Lipid products of PI3K can activate the small GTP-binding proteins RhoA, Rac1 and Cdc42 and lead to actin remodelling, which is important for viral endocytosis (Li *et al.*, 1998a). These data

suggest a positive feedback loop between integrins, PI3K and actin to promote virus uptake (Fig. 3A).

The uptake of HAdV-2/5 is controlled by the large GTPase dynamin (Wang *et al.*, 1998, Meier *et al.*, 2002, Gastaldelli *et al.*, 2008). Recent proteomics analyses have shown that HAdV-5 entry leads to S-nitrosylation of dynamin-2 at cysteine residues C86 and C607, and that the overexpression of S-nitrosylation-resistant C86A or C607A dynamin mutants reduces HAdV infection (Wang *et al.*, 2012). S-nitrosylation is likely mediated by eNOS (endothelial nitric oxid synthase), which gets activated by denitrosylation and phosphorylation upon HAdV-5 entry (Fig. 3A). It remains unknown, however, how HAdV activates eNOS and increases nitric oxide (NO) in epithelial cells.

Signalling to increase receptor availability

The respiratory, digestive, urogenital and ocular tracts are major portals of entry for viruses into vertebrates. Polarized epithelial cells provide the borders for tissues, typically with their apical plasma membrane facing the outside. Intriguingly, many receptors for viruses are not localized on the apical membrane, but at the basal and lateral membranes. This renders polarized cells resistant against infections, for example by HSV-1, poliovirus, reovirus or HAdV (Greber *et al.*, 2007, Bergelson, 2009). The reason why these viruses have no apical receptors is unclear. One can speculate that it is due to protection of the apical membrane by a mucus layer, which precludes the accessibility of the apical plasma membrane to agents in the lumen of the respiratory tracts, for example.

HAdVs invade the lumen of the digestive and respiratory tracts. Their receptors CAR, DSG-2 and integrins are localized at the basolateral membranes. In the airway

lumen, alveolar macrophages engulf incoming viruses. This triggers the release of chemotactic and pro- and anti-inflammatory cytokines (Zsengeller *et al.*, 2000, Higginbotham *et al.*, 2002). The released cytokines include junction-remodelling factors, such as interferon (IFN) γ and tissue necrosis factor alpha (TNF-α). Junctional remodelling by cytokines may facilitate the passage of activated immune cells across the epithelial border (Zen *et al.*, 2005).

A recent study has shown that human blood derived macrophages inoculated with HAdV-2 release IFN-γ, TNF-α or interleukin 6 (IL-6), and also the chemokine IL-8 (Lutschg *et al.*, 2011). When polarized epithelial airway cells were co-cultured with macrophages, they become susceptible to apical HAdV-2. Susceptibility depends to a large extent on IL-8 released from the macrophages. The treatment of polarized epithelial cells with IL-8 was sufficient to induce the expression of CAR and $\alpha_v\beta_3$ integrins on the apical surface, while IFN-γ had no effects (see Fig. 3A). This suggests that CAR and $\alpha_v\beta_3$ integrins are associated with cell migration and immune signalling. IL-8 strongly enhanced apical infection of polarized cells by signalling through the G-protein coupled chemokine receptors CXCR1/2, thereby activating the non-receptor tyrosine kinase Src and the adaptor paxillin. The observation that CAR, which normally engages in homophilic contacts between neighbouring cells, delocalized to the apical membrane under inflammatory conditions suggests that it directly or indirectly coordinates inflammatory responses (Verdino *et al.*, 2010). One can speculate that those HAdV, which elicit low levels of inflammatory responses, are less dependent on cytokines for epithelial infections. These viruses might have found ways to infect epithelial cells by using apical attachment factors, such as CD46, particular lipids, sugars or bridging factors (see also Fig. 1).

Reverse signalling

Virus entry involves a two-way dialogue between the virus and the cell, and comprises forward signalling from the virus to the cell and reverse signalling from the cell to the virus. Already, directly upon AdV attachment to the cell, the particle starts moving on the cell surface. These CAR-dependent motions comprise rapid diffusive motions and steady acto-myosin-dependent drifts (Burckhardt *et al.*, 2011). The drifts are based on actin filaments moving retrograde from the periphery to the cell body. They are driven by actin polymerisation at the periphery and pulling forces from myosin-2 (for a review, see Burckhardt *et al.*, 2009).

The drifting motions of CAR are of key importance for triggering initial steps of the stepwise virus-uncoating program, which starts with the release of the fibers from the virus (Fig. 3B, upper panel). This is assisted by another reverse signal, the binding of $\alpha_v\beta_{3/5}$ integrins to the virus penton base (Burckhardt *et al.*, 2011). Unlike CAR, integrins are static receptors and their motions at the surface are confined. If CAR-induced movements and integrin-induced confinements occur simultaneously at one virus particle, the resulting force on the virus might contribute to loosening the virus structure. This loosening could be enhanced by the interaction of the RGD-loops from penton base with integrins. The latter lead to a clockwise rotation of the penton base pentamer (Lindert *et al.*, 2009). These opposed forces applied to the virus at the cell surface also enable the exposure of protein VI from the inside of the virus. During later steps of infection this can enhance the hydrophobic surface of the virus to break open the limiting endosomal membrane (Wiethoff *et al.*, 2005, Burckhardt *et al.*, 2011).

Upon endosomal escape, the AdV particle is transported via dynein along microtubules towards the perinuclear region. This process was recently reviewed (Dodding *et al.*, 2011, Scherer *et al.*, 2011). Another event of reverse signalling

occurs at one of the last steps of the HAdV-2/5 uncoating program, the disruption of the capsid and the dissociation of the viral DNA from the capsid (see Fig. 3B, lower panel). This takes place at the nuclear pore complex (NPC) (Trotman *et al.*, 2001), and requires the microtubule motor Kif5C (Strunze *et al.*, 2011). Upon docking at the nucleoporin (Nup) 214, the virus recruits conventional kinesin by binding to the Kif5C light chain Klc1/2 (Strunze *et al.*, 2011). The heavy chain is pre-attached to Nup358 at the NPC. Kif5C binding to Nup358 stabilizes the open conformation and leads to allosteric motor activation (Cho *et al.*, 2009). For virus disruption, Kif5C is thought to exert a force on the capsid against a holding force from the NPC. This leads to the separation of the viral DNA from the capsid, and kinesin motors and virus capsid fragments move from the NPC to the cell periphery (Strunze *et al.*, 2011). Now the DNA in association with protein VII but not protein V can be imported into the nucleus, which involves nuclear import factors, such as transportin, importin beta and importin 7 (Trotman *et al.*, 2001, Hindley *et al.*, 2007). This is an example for a direct back-signalling process from the cell to the virus with consequences for virus and NPC integrity. It ensures that the uncoated DNA can readily access the nuclear translocation machinery. We anticipate that other back-signalling events take place on endosomal or cytosolic AdV particles, and possibly also non-related viruses.

Conclusions and outlook to signalling for innate responses

It has been well established that incoming HAdV trigger signal transduction pathways leading to cell activation and innate immunity. The latter processes are less well characterized than the cell activation signals (Fejer *et al.*, 2011). For example, binding of the HAdV-5 fiber to CAR on epithelial cells leads to NFκB activation and enhances the transcription of the chemokines IL-8, GRO-α, GRO-γ or RANTES (Tamanini *et al.*, 2006). p42/44-ERK or p38 mitogen-activated protein kinase appear not to be involved, but HAdV DNA and particular sequence motifs therein are

decoded in endosomes by Toll-like receptor 9 (TLR9) leading to innate signalling (Iacobelli-Martinez *et al.*, 2007, Perreau *et al.*, 2012).

The rupture of endosomal membranes and residence of viruses in the cytosol also contribute to innate immunity signalling. This was suggested from experiments with the HAdV-2 mutant ts1, which is unable to break endosomal membranes and therefore cannot reach the cytosol (Tibbles *et al.*, 2002, Imelli *et al.*, 2009, Smith *et al.*, 2011, Maier *et al.*, 2012). Various cytosolic sensors have been implicated in innate signalling during AdV entry (Fejer *et al.*, 2008). The nucleotide oligomerization domain (NOD)-like receptor (NLR) containing pyrin domain 3 (NLRP3) triggers an inflammasome-dependent response, leading to activation of the IL-1 receptor, NFκB and chemokine release (Barlan *et al.*, 2011). Furthermore, NOD2, another NLR exclusively expressed in monocytes, macrophages, dendritic cells, and intestinal Paneth cells were shown to be involved in innate immune response to HAdV vectors (McDermott *et al.*, 2007, Suzuki *et al.*, 2011). In a previous study it was already demonstrated that NOD2 responds to single strand RNA (Sabbah *et al.*, 2009). Absent In Melanoma (AIM) 2 and the helicase DDX41 are involved in activation of the IFN response factor 3 (Stein *et al.*, 2012). To antagonize an anti-viral state, the immediate early trans-activator E1A of HAdV-5 binds to and dissociates the hBre1/ RNF20 ubiquitin ligase complex, thus inhibiting mono-ubiquitination and inducing the suppression of interferon-stimulated genes (Fonseca *et al.*, 2012). It will now be interesting to investigate, how modulations of host anti-virus responses are integrated in the overall host transcriptional response to HAdV infections. This may lead to a better understanding of both anti- and pro-viral host response pathways.

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Legends to figures

Fig. 1: Summary of HAdV entry receptors, attachment- and bridging factors and tropism.

Additional information and literature can be found in the main text.

Fig. 2: Signalling events during HAdV-B entry into non-polarized epithelial or hematopoietic cells.

Binding of HAdV-3/35 to its receptors CD46 and αvβ3/5 integrins leads to the activation of Rac1, which is involved in actin remodelling and results in membrane curvatures termed 'wobbling' (Amstutz *et al.*, 2008, Kalin *et al.*, 2010). Furthermore, Pak1 and CtBP1 are activated in a Rac1 dependent way by HAdV-B, and lead to macropinocytosis. The virus-containing macropinosomes are positive for Pak1 and CtBP1. Possibly, this entry pathway interferes with innate immunity responses through the transcriptional co-repressor CtBP1.

Fig. 3: Forward and reverse signalling in cell entry of HAdV.

A. Forward signalling and receptor accessibility.

HAdV-2/5 can be taken up by macrophages that 'patrol' on the apical side of polarized epithelial cells. This leads to the secretion of various cytokines including interleukin 8 (IL8). IL8 binds to the CXCR1/2 receptor and triggers the phosphorylation (P) of the non-receptor tyrosine kinase Src and the adaptor paxillin. These proteins are involved in relocating CAR and integrins from the basal to the apical surface and thus enable virus attachment and infection from the apical plasma

membrane (Lutschg *et al.*, 2011). The binding of HAdV-2/5 to integrins leads to numerous intracellular signals, including PI3K and the actin remodelling GTPases RhoA, Cdc42 and Rac, and promote virus endocytosis (see main text). HAdV-2/5 endocytosis is controlled by dynamin-2 (Dyn2), which gets S-nitrosylated (NO) most likely by the endothelial nitric oxid synthase (eNOS) (Wang *et al.*, 2012). This pathway leads to infection.

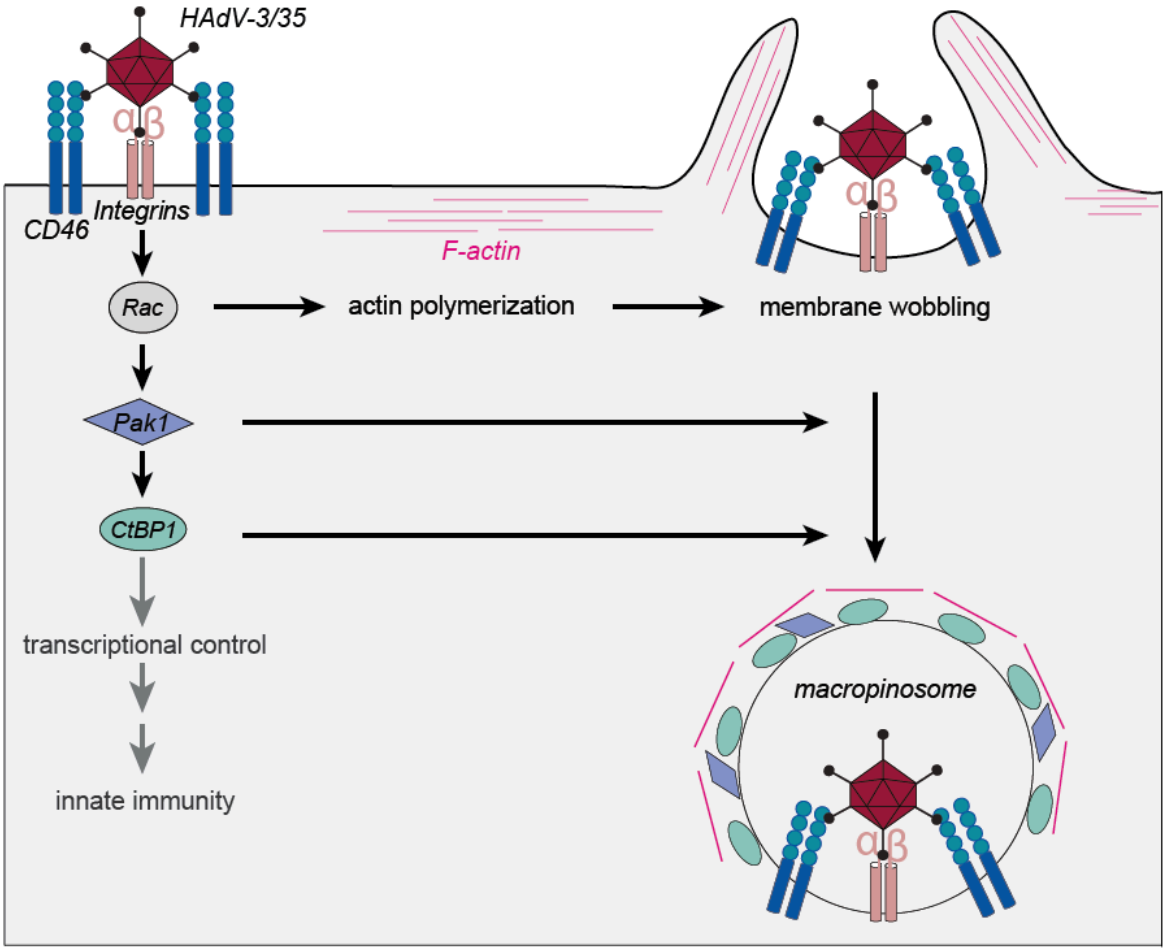
B. Reverse signalling.

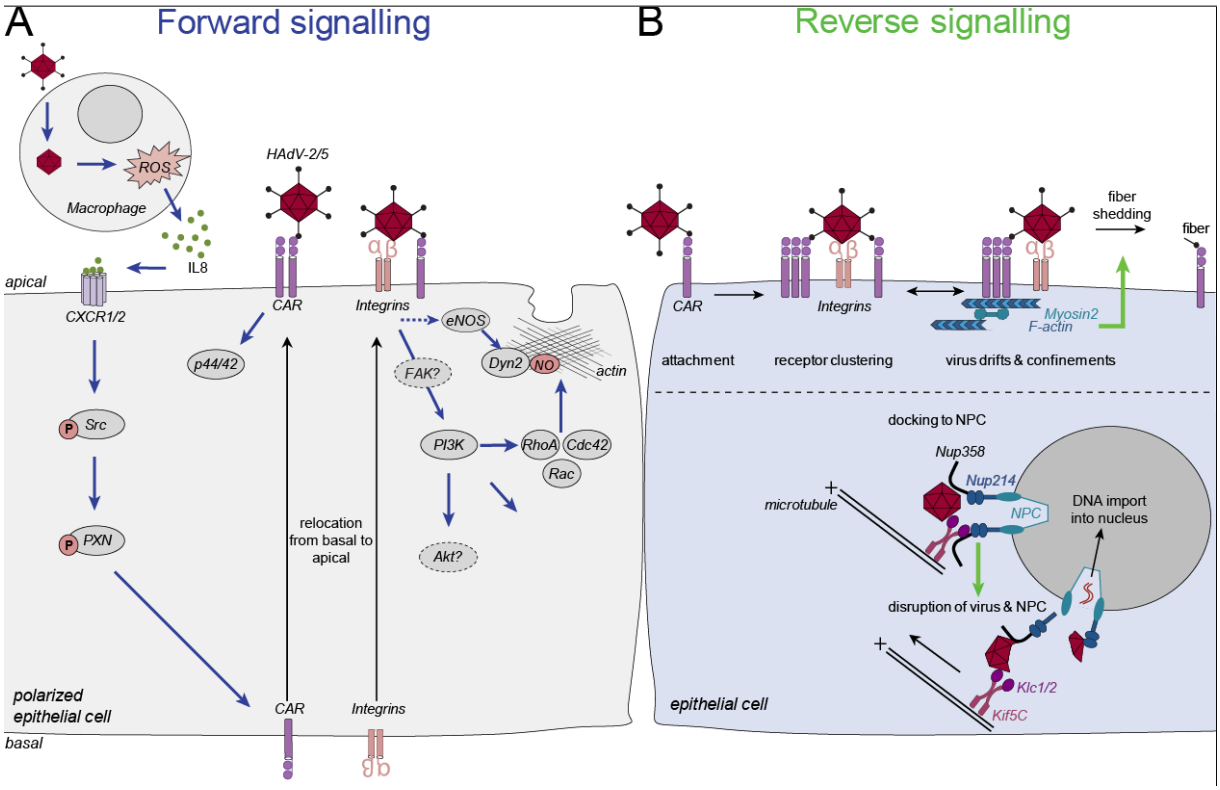
Reverse signals from the cell to the virus emerge for example, when myosin2 and actin-dependant movements of CAR are attenuated by immobile integrins. This creates a force on the virus, and leads to loosening of the capsid structure, as measured by the loss of fibers (upper part) (Burckhardt *et al.*, 2011). Additional reverse signals from the cell to the virus may come from cytoplasmic motors, such as dynein/dynactin and result in displacement of the cytosolic virus towards the nucleus. We do not show these events, since they have been covered in recent reviews (Greber *et al.*, 2006, Dodding *et al.*, 2011, Scherer *et al.*, 2011). During later steps of infection the virus docks to the nuclear pore complex (NPC) by interacting with the nucleoporin (Nup) 214, and subsequently binds to the light chain (Klc1/2) of the conventional kinesin Kif5C (Strunze *et al.*, 2011). The movement of the motor towards the microtubule plus end occurs against a holding force from the NPC, and leads to the disruption of the capsid and in part the NPC. This facilitates the release of the viral DNA into the nucleus (lower part).

	Attachment factors					Entry receptor	Bridging factors			
Tropism	**CAR**	**CD46**	**DSG2**	**SA**	**GD1a**	**Integrins**	**HSPG**	**FX**	**DPPC**	**Lf**
Respiratory	2, 4, 5, 12, 15, 19p, 31	3, 7, 14, 16, 21, 50	3, 7, 14			2, 3, 5, 35	2, 3, 5		2, 5	2, 5
Ocular	5, 9	37, 49		8, 19a, 37	8, 19a, 37					
Renal		11, 35	11, 14, 35							
Intestinal	41									
Liver							2, 3, 4, 5, 6, 7, 8	2, 5, 17, 24, 30, 33, 45, 47		

Coxsackie and adenovirus receptor (CAR); Desmoglein 2 (DSG2); Sialic acid (SA); Ganglioside 1a (GD1a); Heparan sulfate proteoglycan (HSPG); Coagulation factor X (FX); Dipalmitoyl phosphatidylcholine (DPPC); Lactoferrin (Lf)

Colour-code for the adenovirus (sub)-species: A, B1, B2, C, D, E, F


HAdV-3/35
αβ
CD46
Integrins
F-actin
αβ
Rac
actin polymerization
membrane wobbling
Pak1
CtBP1
transcriptional control
innate immunity
macropinosome
αβ


A
Forward signalling
B
Reverse signalling
ROS
Macrophage
IL8
HAdV-2/5
apical
CXCR1/2
CAR
Integrins
eNOS
p44/42
FAK?
Dyn2
NO
actin
Src
PI3K
RhoA
Cdc42
Rac
PXN
Akt?
relocation from basal to apical
polarized epithelial cell
basal
CAR
Integrins
fiber shedding
fiber
Myosin2
F-actin
attachment
receptor clustering
virus drifts & confinements
docking to NPC
Nup358
Nup214
NPC
microtubule
DNA import into nucleus
disruption of virus & NPC
Klc1/2
Kif5C
epithelial cell