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Parvovirus particles and movement in the cellular cytoplasm and effects of the cytoskeleton

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ABSTRACT

Cell infection by parvoviruses requires that capsids be delivered from outside the cell to the cytoplasm, followed by genome trafficking to the nucleus. Here we microinject capsids into cells that lack receptors and followed their movements within the cell over time. In general the capsids remained close to the positions where they were injected, and most particles did not move to the vicinity of or enter the nucleus. When 70 kDa-dextran was injected along with the capsids that did not enter the nucleus in significant amounts. Capsids conjugated to peptides containing the SV40 large T-antigen nuclear localization signal remained in the cytoplasm, although bovine serum albumen conjugated to the same peptide entered the nucleus rapidly. No effects of disruption of microfilaments, intermediate filaments, or microtubules on the distribution of the capsids were observed. These results suggest that movement of intact capsids within cells is primarily associated with passive processes.

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Introduction

Cell infection by viruses that replicate in the nucleus involves viral components being delivered into the cytoplasm and then transfer of the genome to the nucleus, generally along with viral proteins or capsid components (Greber and Fornerod, 2005; Marsh and Helenius, 2006). The processing or transport of infecting capsids or nucleocapsids within the cytoplasm, and the transport of the genome to the vicinity of or into the nucleus can be complex as the cytoplasm prevents the free diffusion of virus-sized particles (Lukacs et al., 2000; Seksek et al., 1997). For adenoviruses, herpesviruses, and at least some retroviruses, viral proteins and structures are actively transported within the cytoplasm to the vicinity of the nucleus (Lagache et al., 2009b), while for other viruses, including papillomaviruses and polyomaviruses endosomal mechanisms are used to transport the capsids to the endoplasmic reticulum or other compartments (Engel et al., 2011; Gruenberg, 2009; Sapp and Bienkowska-Haba, 2009).

The capsids of parvoviruses or adeno-associated viruses (AAVs) bind receptors on the cell surface, enter the cells by receptor-mediated endocytosis, and then traffic within endosomes to the microtubular organizing center (MTOC) (Ding et al., 2005; Harbison et al., 2008; Harbison et al., 2009; Vendeville et al., 2009). Release from endosomes appears to be quite slow and requires the activity of a phospholipase A_2 in the unique region of the viral protein 1 (VP1), and many parvoviral capsids are retained within endosomes for up to several hours (Farr et al., 2005; Zadori et al., 2001). Expression of PLA_2 in cells can alter the cellular morphology (Deng et al., 2013). Because of the slow release of the capsids, in studies of viral entry it can be difficult to know whether caspids being detected are within the cytoplasm or endosomes.

The roles of the different cytoskeleton elements in viral infection appear to be complex. In some studies infection has been shown to depend on the presence of an intact microtubular cytoskeleton, and capsids of autonomous parvoviruses (canine parvovirus (CPV) and porcine parvovirus), and at least some adeno-associated viruses (AAVs) have been suggested to be trafficked within the cytoplasm in association with the molecular motor dynein (Kelkar et al., 2006, 2004; Suikkanen et al., 2003a). Addition of peptides to AAV type-2 capsids that were predicted to bind dynein light chain (LC8) also enhanced retrograde transport in axons (Xu et al., 2005). However, other studies have suggested that an intact cytoskeleton is less important for cell infection (Hirosue et al., 2007), and it is unclear whether cytoplasmic trafficking of parvovirus capsids is an active trafficking mechanism, occurs by diffusion, or involves some combination of those

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processes. A role of intermediate filaments and vimentin in infection by the MVM parvovirus has been reported in localization of virions around the nucleus, and the filaments became rearranged in cells that have taken up virions from the cell surface and in many infected cells (Fay and Pante, 2013). After cells are infected there may be extensive changes in the cellular architecture that result from virus replication and expression of the viral NS1 protein (Nuesch et al., 2005).

When free in the cytoplasm parvovirus capsids may become conjugated to ubiquitin, and in some cases the capsid proteins are degraded by proteosomal systems (Boisvert et al., 2010; Ros and Kempf, 2004; Yan et al., 2002). However, the effects reported vary for different viruses, and while proteosomal inhibitors such as MG138 enhance transduction by AAV type-2 or type-5 (Ding et al., 2003; Yan et al., 2004), they inhibit infection by autonomous parvoviruses (Ros and Kempf, 2004), and it may be difficult to distinguish direct and indirect effects of the drugs. AAV2 capsids may also be modified by ubiquitin addition to surface exposed tyrosines (Tyr), and mutating one or more of the several Tyr on the capsid surface can enhance transduction due to alterations that capsid modification (Zhong et al., 2008a; Zhong et al., 2008b).

The processes of nuclear entry and exit of parvovirus capsids are still not understood in detail, and may vary between viruses and perhaps cell types. When capsids of autonomous parvoviruses or AAV2 enter cells by receptor-mediated endosomal processes only a low proportion are seen to enter the nucleus by microscopy (Bantel-Schaal et al., 2002; Harbison et al., 2009; Seisenberger et al., 2001). Purified CPV capsids microinjected into the cytoplasm of cells and detected after cell fixation remained in the cytoplasm for more than 2 h (Vihinen-Ranta et al., 2002, 2000). Several AAV serotypes may infect cells more rapidly, and higher proportions were recovered in the nuclear-associated fractions a few hours after uptake from the cell surface (Sonntag et al., 2006; Zhong et al., 2008b). Both endosomal release and nuclear transport have been associated with the release or exposure of the N-termini of some or all of the 5 or 6 VP1s in each capsid, which contain both the phospholipase A_2 enzyme activity and sequences made up of basic amino acids, similar to classic nuclear localization sequences (NLS) (Sonntag et al., 2006; Vihinen-Ranta et al., 2002). The VP1 unique regions are usually sequestered within the capsid but become exposed in the endosome (Farr and Tattersall, 2004; Suikkanen et al., 2003b), and some would be on the outside of capsids that enter the cytoplasm (Vihinen-Ranta et al., 2002).

After replication parvovirus capsids assemble in the nucleus, and in many cases appear to be retained there. However, capsids may also be trafficked to the cytoplasm or even out of the cell. The export of intact newly produced minute virus of mice (MVM) capsids out of the nucleus occurred efficiently for some virus-cell combinations, and was regulated by the phosphorylation of Ser and Thr within the N-terminal sequence of VP2 exposed on the outside of the newly produced full capsids (Maroto et al., 2004). The process of nuclear export and cytoplasmic and extra-cellular transport of the capsids was also associated with remodeling of the actin cytoskeleton by the enzyme gelsolin, which is modified by the viral NS1 protein (Bar et al., 2008).

Capsids in the cytoplasm are reported to directly alter the structure of the nuclear envelope. When MVM capsids were injected into the cytoplasm of *Xenopus* oocytes, they changed the integrity and morphology of the nuclear envelope as seen by electron microscopy, with damage particularly to the outer nuclear membrane (Au et al., 2010; Cohen and Pante, 2005). When purified capsids were added to digitonin-permeabilized fibroblasts the nuclear envelope morphology changed to show ruffles and patches, as detected by staining for Lamin A/C (Cohen et al., 2006). The nuclear breakdown was reported to involve enzymes involved in nuclear changes that occur during mitosis, including protein kinase C which acted on cdk-2, and which was acted on by caspase-3 (Porwal et al., 2013).

Here we further examine the intracellular trafficking of parvoviral capsids by examining the distribution of capsids within the cytoplasm of live cells after microinjection. Fluorescently labeled capsids generally remained close to the location where they were injected, with little movement even over periods of hours, and similar effects were seen with unlabeled capsids. That localization was not significantly altered by changes in the structures of the microfilaments or microtubules, or by the presence of intermediate filaments.

Results

Here we examined the locations and movement of fluorescently labeled virus capsids after injection into live cells, and compared the results to those seen for unlabeled capsids in cells that were fixed after various times of incubation. Capsids were labeled with Alexa488 or Alexa594 and were in the form of single particles as described previously (Harbison et al., 2009). Initial studies showed no obvious differences in the distribution or movement of full or empty capsids (results not shown), and most studies were conducted with full particles, which represent the infectious virions.

We initially examined the distribution of capsids in feline NLFK cells which express feline transferrin receptor type-1 (TfR) and which bind and endocytose the virus, as well as within TRVb cells, which are derived from CHO cells and which lack the TfR and which do not bind viruses. The latter cells are efficiently infected when expressing the feline TfR from a plasmid (Goodman et al., 2010; Parker et al., 2001), indicating that the cytoplasmic and nuclear transport processes required for infection are functional. When capsids were released from the microinjection needle near NLFK cells some were subsequently found on the surface and filopodia of the cells, indicating that they bound receptors and were endocytosed (Fig. 1A), so that using those cells would show both injected and endocytosed capsids. However, receptor-negative TRVb cells showed no evidence of surface bound virus under the same conditions (Fig. 1B), and those were therefore used for the remainder of these studies.

When fluorescently labeled CPV capsids were injected into the cytoplasm of live NLFK or TRVb cells they remained near the position of injection for between 2 min and 1 h, with little movement being observed (Fig. 1A and B). Twenty nanometer diameter Cy5-labeled polystyrene nanospheres injected into TRVb cells also showed little movement within the cytoplasm (Fig. 1C).

Previous studies have indicated that parvovirus capsids in the cytoplasm can alter the nuclear morphology and nuclear envelope integrity (Au et al., 2010; Cohen et al., 2006; Cohen and Pante, 2005). Cells expressing Lamin A/C-GFP were injected with Alexa594-labeled full capsids (Fig. 2A) or with unlabeled capsids (Fig. 3A,B), and examined using widefield and confocal microscopy. The capsids remained near where they were injected (Fig. 2A) with only a small proportion becoming localized adjacent to the nuclear envelope (Figs. 2A and 3A,B). Some of the virus-injected cells showed subtle changes in the distribution of Lamin A/C that were not seen in the non-injected cells or in cells injected with labeled BSA (Fig. 2B). When Alexa488-70 kDa dextran was injected into the cytoplasm it mostly remained in that location after 1.5–2 h with limited movement into the nucleus (Fig. 4A). When injected along with capsids the 70 kDa dextran entered the nucleus at similar rates as in the absence of capsids (Fig. 4B).

Capsids were conjugated to NLS peptides, with an average of 5 or 28 peptides attached to each capsid in two different conjugated preparations. Peptide conjugated capsids were injected

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