Welcome to the nucleus: CAN I take your coat?

Amnon Harel and Douglass J. Forbes

Individual viruses have evolved strategies for surmounting a formidable barrier in their path to replication in the nucleus — the nuclear envelope. A new study describing the nuclear entry of adenovirus 2 finds that this virus docks at the CAN/Nup214 protein of the nuclear pore, then hijacks histone H1 and specific H1-import receptors to effect a targeted uncoating of its nucleocapsid at the nuclear pore.

Many infectious viruses, from herpes to hepatitis, SV40 to human immunodeficiency virus (HIV), replicate in the cell nucleus[1]. Adenovirus, with its double-stranded DNA genome, is of particular interest because of its use as a gene therapy vector. Following infection, an adenovirus particle undergoes a series of changes in the cytoplasm which weaken the particle but leave the nucleocapsid intact, with a diameter of 60–90 nm. The nucleocapsid 'fast tracks' to the exterior of the nucleus using microtubules and molecular motors (Fig. 1). The nuclear pores, with a maximum expandable channel diameter of ≤ 40 nm (ref. 2), constitute the next hurdle to adenovirus infectivity.

It has been suggested that viruses with an obligatory nuclear replication step contain proteins with nuclear localization signals (NLSs). Such NLSs are thought to engage cellular nuclear import receptors, such as the importins α and β. These then bind to the nuclear pore and translocate either the viral nucleocapsid (if it is small and/or deformable) or the viral genome (if the nucleocapsid is too large or unwieldy) into the nucleus. Experimental evidence supporting an import-receptor-mediated model exists for a handful of viruses[1]. The HIV preintegration complex operates differently, in that it contains proteins such as Vpr, which bind directly to the nuclear pore[3–4], abrogating the need for cellular import receptors.

The nucleocapsids of large viruses such as adenovirus and herpes virus cannot physically pass through the maximally dilated channel (around 40 nm diameter)[2] of the large nuclear pore complex (150 nm diameter; molecular mass 120 million)[5]. Instead, these viral nucleocapsids dock at the pore and inject their genomes[1,6]. On page 1092 of this issue[7], Greber and colleagues report that adenovirus 2 (Ad2) binds specifically to a protein on the cytoplasmic filaments of the nuclear pore — the nucleoporin Nup214 or CAN (Fig. 2). Strikingly, antibodies to CAN/Nup214 block the binding of Ad2 to isolated nuclear envelopes. Antibodies to other nucleoporins have no effect. Neither cytosol nor import receptors, such as importins α and β, are required for this binding, implying a direct interaction between CAN/Nup214 and the viral nucleocapsid. CAN/Nup214 has not previously been seen to bind import cargo, but instead serves as a terminal binding site for export receptors on their way out of the nucleus. Import receptors have been thought to interact instead with the nucleoporin Nup358 when they first contact the pore[8]. A report by Nishida and colleagues[9], however, implicates CAN/Nup214 in the nuclear import of MAP kinase.

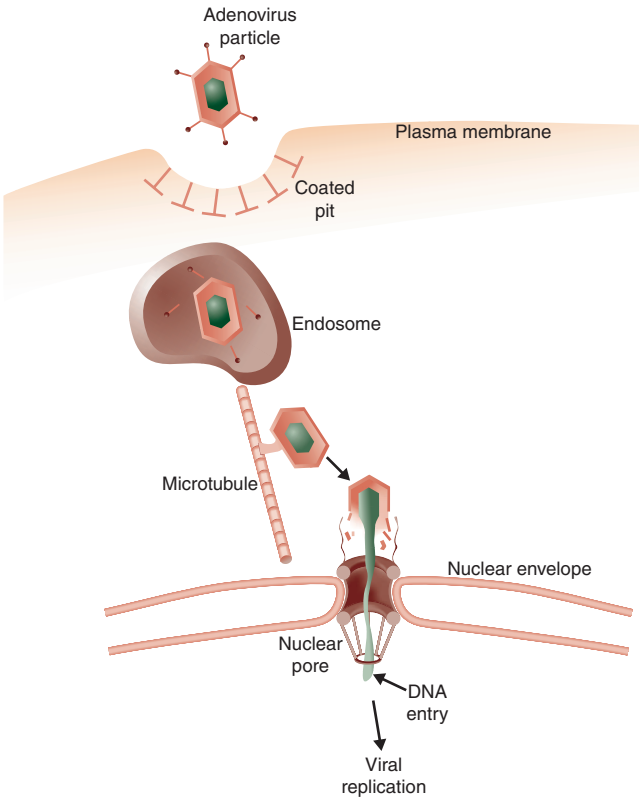

Figure 1 **Schematic of entry of adenovirus into the cell and uncoating (see text for details).**

To ask whether Nup214 has a pivotal role in Ad2 infection *in vivo*, epithelial cells were infected with Texas Red-labelled Ad2 particles and examined at different times[7]. To follow progression of the infection, an antibody that recognizes the adenovirus hexon protein only in nucleocapsids that have begun disassembly was used. Intact Texas Red-labelled viral particles were observed early on, but by 120 min after

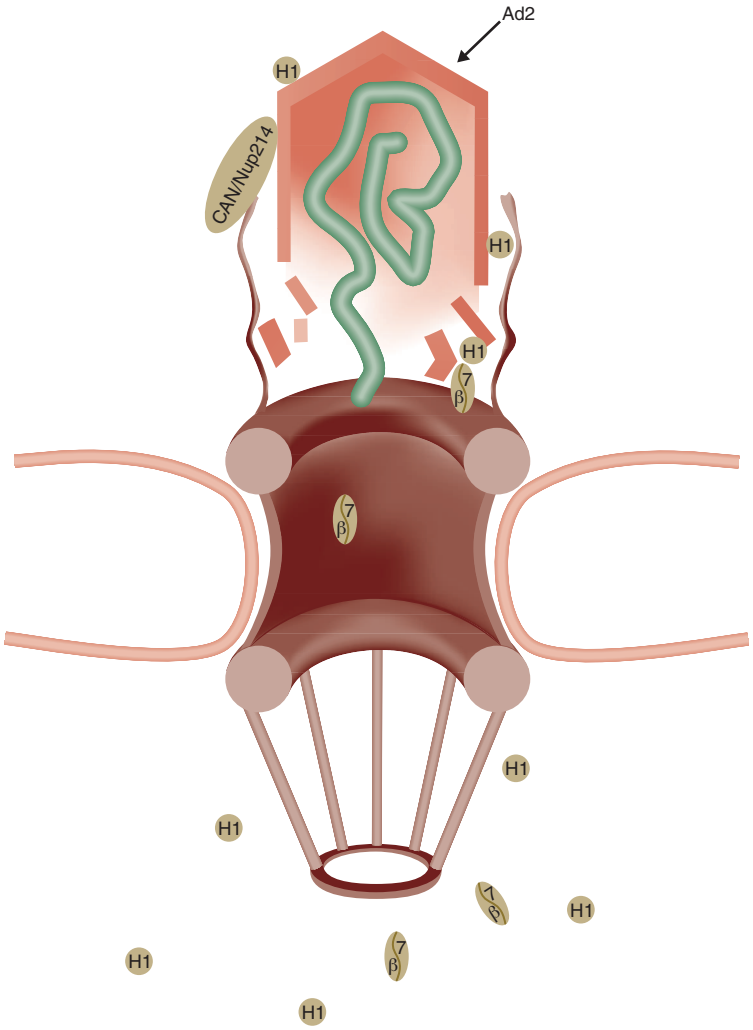

Figure 2 **Model for nuclear entry of Ad2. The Ad2 particle docks on the cytoplasmic fibrils of the nuclear pore by binding CAN/Nup214. Small amounts of histone H1 escape from the nucleus and bind to hexon protein on the proximal side of the docked capsid. Importin β–importin 7 dimers bind to H1, inducing import of the proximal H1–hexon complexes and triggering capsid disassembly. Consequently, the viral DNA is liberated near the opening of the pore and positioned for translocation into the nucleus. Figure adapted from ref. 7.**

infection many had converted to antibody-accessible nucleocapsids at or near the nucleus. Capsid disassembly could be blocked entirely by antibodies against CAN/Nup214, implying that binding to CAN precedes the initiation of disassembly. Moreover, the entry of Ad2 DNA into the nucleus, assayed by fluorescence *in situ* hybridization (FISH), was also blocked by the anti-CAN antibodies.

In a search for the proteins required subsequent to CAN-mediated binding to the nuclear pore, Greber and colleagues[7] found that Ad2 binds specifically to two proteins present in a low-salt extract of nuclear envelopes. Surprisingly, these are histone H1.2 and a second histone H1 variant. These apparently bind to the abundant hexon protein of the nucleocaspid. Cementing a role for histone H1 in the process, nuclear microinjection of antibodies to H1 blocked both capsid disassembly and nuclear entry of Ad2 DNA. Injection of antibodies to other histones or to a phosphorylated H1 had no effect. Anti-H1 antibodies did not, however, block the pore-docking step. The authors speculate that H1 binds to an acidic cluster on the surface of the hexon protein, a cluster characteristic of the Ad2 and Ad5 viral subgroups. An Ad3 relative lacks this hexon sequence motif and its nuclear entry is unaffected by anti-H1 antibodies. Previous studies have shown that newly synthesized histone H1 is imported into the nucleus by a heterodimeric import receptor consisting of importin β and importin 7 (ref. 10). In a striking proof of postulate[7], antibodies to either of the H1-import-receptor subunits were found to block Ad2 capsid disassembly and viral DNA entry into the nucleus.

A two-step model proposed by Greber and colleagues encapsulates their nuclear targeting and DNA entry results[7]. First, viral particles stably dock on the cytoplasmic fibrils of the nuclear pore through a direct interaction with CAN/Nup214. Then, small amounts of histone H1 escape from a dynamic nuclear pool of H1 and preferentially bind to the proximal side of the docked adenovirus nucleocapsid. This, in turn, attracts the importin β–importin 7 heterodimer, inducing binding and import of the proximal H1–hexon complexes and in effect, causing localized capsid disassembly (Fig. 2). Such 'targeted disassembly' is proposed to liberate and position the viral DNA for directed release into the nucleus.

The most poorly understood stage of adenoviral infection remains the translocation of DNA through the nuclear pore. Recently, Elbaum and colleagues[11] used an elegant optical tweezers approach *in vitro* to follow the path of individual double-stranded DNA molecules, in their case derived from bacteriophage λ, into the nucleus. DNA molecules of 50 kilobases were tagged at one end with an NLS and at the other with a large polystyrene bead. By following the bead, passage of single molecules of DNA through the pore could be observed in real time and the kinetics of entry analysed mathematically to determine the mechanism. DNA translocation occurred in a manner consistent with linear diffusion through the pore. Specifically, the DNA tether holding the bead to the nucleus shortened with time in a manner characteristic of diffusion and independent of energy hydrolysis. This was followed by an irreversible retention of the DNA within the nucleus, most likely through chromatin formation[11]. The presence of NLS peptides at one end of the double-stranded DNA greatly facilitated initial association with the nuclear pore, but had no effect on the kinetics of DNA entry. This system can be viewed as a simplified model for the entry of viral DNA. Once launched, by targeted capsid disassembly, Ad2 DNA might similarly diffuse passively through the pore. Alternatively, proteins coating the Ad2 DNA may engage additional import receptors which interact with different nucleoporins further down the nuclear pore channel.

Previous work[12] had invoked importins α and β, as well as the chaperone protein Hsc70, in the nuclear entry of Ad2. The

significance of the new work[7] is in shifting our attention first to a docking step on the pore fibrils, which does not require importin β or other factors, and then to an unexpected role for nuclear histone H1 and importin β–importin 7 in capsid disassembly and DNA entry. Discrepancies between these studies[7,12] could be explained if the requirement for importin β in the permeabilized-cell study[12] actually reflects a requirement for importin β downstream of the docking step, or if it reflects differences between the *in vivo*[7] and permeabilized cell[12] systems. Future work is likely to reveal requirements for additional cytosolic factors, as well as additional steps that can be experimentally inhibited.

The use of importins in capsid disassembly[7] has certain parallels with the recent exciting discovery that importins, in addition to acting as import receptors, can act as cellular switches that spatially control microtubule and spindle assembly (reviewed in refs 5, 13–14). In mitotic cells, for example, free importins α and β bind to and inhibit proteins required for spindle assembly. This inhibition of spindle assembly is, however, turned off in the vicinity of the mitotic chromosomes. There, Ran–GTP (produced by chromosomally bound Ran guanine-nucleotide exchange factor) locally disrupts the inhibitory complexes and allows spindle assembly. Thus, the adenovirus study of Greber and colleagues[7] is impressive not only for revealing a distinct viral entry pathway, but also as a novel example of how the highly abundant importins (~2 μM in *Xenopus* eggs) can be used to control different cellular activities. □

Amnon Harel and Douglass J. Forbes are in the Section of Cell and Developmental Biology, Division of Biology 0347, University of California, San Diego, La Jolla, California 92093-0347, USA
e-mail: dforbes@ucsd.edu

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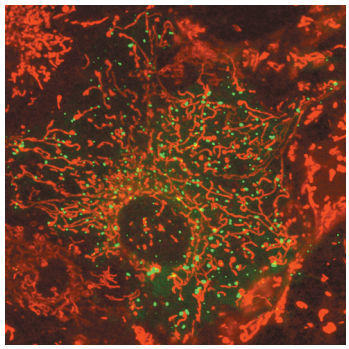
Mitochondrial fission in life and death

Mitochondria must replicate during cell division to ensure that daughter cells inherit roughly as many mitochondria as their mother cell. This is achieved through fission prior to cell division and subsequent mitochondrial enlargement in the daughter cells. Intriguingly, a similar mitochondrial fission phenomenon is observed in apoptotic cell death, where apoptosis is accompanied by fragmentation of subcellular organelles, including mitochondria, and eventually of the cell itself. Now Youle and colleagues shed light on the mechanism of mitochondrial fission in apoptosis, and find that it is indeed similar to the process of mitochondrial division (*Dev. Cell* **1**, 515–525 (2001)). They find that the dynamin-related protein Drp1 translocates from the cytosol to mitochondria after induction of apoptosis (see figure, Drp1 is stained in green and mitochondria in red).

Dynamin proteins function in membrane constriction by virtue of their mechanochemical properties. When Youle and colleagues overexpressed a dominant-negative mutant form of Drp1 (DN Drp1), it inhibited mitochondrial fragmentation in apoptotic cells. Interestingly, a protein homologous to Drp1, Dnm1, has been implicated in mitochondrial division in yeast.

In contrast to DN Drp1, overexpression of Bax, a protein that acts to induce apoptosis in a mitochondria-dependent manner, increases mitochondrial fragmentation. The BH3 domain of Bax is required for this fragmentation; BH3 is also essential for the pro-apoptotic activity of Bax. The Youle laboratory found that DN Drp1 blocked Bax-induced mitochondrial fission; and in cells expressing both Bax and DN Drp1, mitochondria seem to be swollen. So it is possible that Drp1 relocalization from the cytosol to mitochondria in apoptotic cells could limit the extent of mitochondrial swelling.

DN Drp1 also prevents the loss of mitochondrial membrane potential and release of cytochrome *c* from the mitochondrial intermembrane space, and indeed cell death itself (as assessed by DNA fragmentation, one of the hallmarks of apoptosis). Mitochondrial swelling, loss of mitochondrial membrane potential, and Bax-like proteins have all been proposed to regulate the release of cytochrome *c* and other death-promoting molecules from mitochondria in apoptotic cells, so it seems that the function of Drp1 could be central to the regulation of cell death. Youle and colleagues even speculate that Drp1 could mediate the formation of vesicles at the outer mitochondrial membrane, which could contribute to the release of pro-apoptotic proteins from the mitochondrial intermembrane space to the cytosol after induction of apoptosis, but that remains to be tested. It also remains to be confirmed whether the function of Drp1 is due to its mechanochemical properties.



Richard Youle

VALERIE FERRIER