

# A model for cofactor use during HIV-1 reverse transcription and nuclear entry<sup>☆</sup>

Laura Hilditch and Greg J Towers

Lentiviruses have evolved to infect and replicate in a variety of cell types *in vivo* whilst avoiding the powerful inhibitory activities of restriction factors or cell autonomous innate immune responses. In this review we offer our opinions on how HIV-1 uses a series of host proteins as cofactors for infection. We present a model that may explain how the capsid protein has a fundamental role in the early part of the viral lifecycle by utilising cyclophilin A (CypA), cleavage and polyadenylation specificity factor-6 (CPSF6), Nup358 and TNPO3 to orchestrate a coordinated process of DNA synthesis, capsid uncoating and integration targeting that evades innate responses and promotes integration into preferred areas of chromatin.

## Addresses

University College London, Medical Research Council Centre for Medical Molecular Virology, Division of Infection and Immunity, University College London, 90 Gower Street, London WC1E 6BT, United Kingdom

Corresponding author: Towers, Greg J ([g.towers@ucl.ac.uk](mailto:g.towers@ucl.ac.uk))

**Current Opinion in Virology** 2014, 4:32–36

This review comes from a themed issue on **Virus entry**

Edited by **Mark Marsh** and **Jane A McKeating**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 14th January 2014

1879-6257/\$ – see front matter, © 2013 The Authors. Published by Elsevier B.V. All rights reserved.

<http://dx.doi.org/10.1016/j.coviro.2013.11.003>

## Introduction

Retroviruses are defined by their ability to integrate a DNA copy of their genome into the host chromatin. In order to achieve this, they must first reverse transcribe (RT) their RNA genome into double stranded DNA and then gain access to the nucleus. The majority of retrovirus families are dependent upon mitosis to access the nuclear compartment. In contrast, lentiviruses, such as human immunodeficiency virus type-1 (HIV-1), have evolved to traverse the nuclear pore complex (NPC) allowing replication in non-dividing cells such as macrophages. Whilst the molecular details of reverse transcription and integration have been well established, much remains

uncertain regarding the loss of the capsid (CA) shell that protects the reverse transcription complex (RTC), a process called uncoating. In particular, the host cell cofactors on which the virus depends have been the subject of intense scrutiny of late, and as a result our understanding is rapidly progressing. It is becoming evident that the early interactions between host cofactors and the viral CA are key in determining the path taken by the viral core, and that these interactions influence downstream processes. As further details become clear we propose that this new knowledge will allow novel therapeutic interventions, and more effective use of lentiviruses as tools.

## The role of CA in early infection

### The timing of uncoating

Uncoating has been one of the most enigmatic aspects of early HIV-1 infection and is defined as the loss of the CA core from the RTC. Conflicts of size suggest that the conical core must be lost before nuclear entry: the width of the core is 50–60 nm, whereas the NPC pore diameter is ~30 nm, but the precise timing and mechanism of uncoating remain undefined. Early biochemical analyses of cores purified from newly infected cells suggested that the HIV-1 CA core is unstable and lost soon after cell entry [1,2]. This was interpreted as CA being dispensable for subsequent viral processes. However, recent genetic data suggest that CA stays associated with the virion for longer [3<sup>•</sup>]. The different models for disassembly stem in part from the fact that biochemical assays measure what happens to the majority of particles, most of which do not successfully infect the cell, whereas genetic analyses can be focused on the infectious particles only, for example, by reading out infection through GFP expression [4]. Microscopy approaches have also led to conflicting conclusions, with some studies supporting cytoplasmic uncoating, whilst others have suggested later, NPC associated, uncoating [5<sup>•</sup>,6–8].

### A functional role for CA

Consistent with an important role for CA in the incoming phase of the life cycle, several studies have functionally linked reverse transcription and uncoating. An increasing number of studies demonstrate that suppressing reverse transcription delays uncoating, supporting a role for reverse transcription in promoting uncoating [5<sup>•</sup>,9,10]. Furthermore, certain CA mutants have defects in reverse transcription [11,12]. Some authors have suggested that a late role for CA, despite early uncoating, may be rationalised by partial CA uncoating in the cytoplasm. However, it is difficult to understand how partial uncoating

<sup>☆</sup> This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

would not lead rapidly to complete uncoating given that the CA structure appears to be unattached to its contents, and dependent on a lattice of CA-CA interactions [13]. However, it is becoming evident that mutations in CA can influence events that occur downstream of the uncoating event. HIV-1 CA has now been well established as a viral determinant for nuclear entry and the ability to infect non-dividing cells [14–16]. As discussed below, mutation of CA also impacts on the use of downstream cofactors including TNPO3, Nup358 and Nup153, all of which influence integration site targeting [3<sup>•</sup>,17,18<sup>•</sup>]. CA is therefore likely to also have consequences for expression of the viral RNA/proteins due to its influence on the provirus context within chromatin.

### Host cofactors for nuclear entry

The fact that CA impacts on viral processes even after its loss most likely results from influential interactions that occur between the CA and host cofactors before the uncoating process begins. How CA has such a central role in the behaviour of HIV-1 is gradually being uncovered by a wealth of literature surrounding the identification and role of host proteins in this stage of HIV infection. As our understanding of these data grows, so it informs our understanding of basic viral and cellular biology.

#### TNPO3/nucleoporins

In 2008 genome wide siRNA screens identified a large number of putative host cofactors for HIV-1 infection [19,20]. Amongst these were the karyopherin TNPO3, also identified as an HIV-1 integrase interactor in a yeast-2-hybrid screen [21], and NPC proteins Nup153 and Nup358. Depletion of these proteins using RNA interference impaired HIV-1 infection [3<sup>•</sup>,17,18<sup>•</sup>,21–23], and also impacted on integration site selection, with integrated proviruses still being identified in genes but in regions of lower gene density [24,25<sup>•</sup>]. The precise step of the viral life cycle at which these cofactors act has been disputed [7,17,18<sup>•</sup>,21,26–31]. This has been largely due to conflicting measurements of the abundance of 2LTR circles, which are formed by components of the non-homologous end joining pathway that are found uniquely in the nucleus. Thus 2LTR circles are a much-used marker for nuclear entry. However, two recent studies elegantly demonstrated that 2LTR circle PCR assays must use primer/probe sequences that actually span the LTR-LTR junction, or they can detect the autointegrants that form as completed RT products back up at the defective NPC and integrate into each other [32<sup>•</sup>,33<sup>•</sup>]. The autointegrants in these studies were identified by sequencing the 2LTR PCR products. The use of appropriately designed primers supports a nuclear entry defect on depletion of TNPO3 [32<sup>•</sup>].

How might HIV-1 CA influence nuclear entry? HIV-1 CA is certainly capable of interacting with the NPC directly

by binding to the cyclophilin-like domain of Nup358 [7,18<sup>•</sup>]. In this way Nup358 recruitment may tether a reverse transcribing virion to the NPC and orchestrate interaction with the nuclear transport machinery during the uncoating process. The isolated Nup358 Cyp domain has been shown to catalyse *cis-trans* isomerisation of the G89-P90 bond in CA using NMR techniques [34] and although it is irresistible to hypothesise that this manipulation of CA has a role in controlling uncoating, direct evidence remains elusive. Nup358 also possesses Ran binding domains, and as such could play a role in the regulation of RanGTP dependent nuclear import. TNPO3, itself a RanGTP dependent nuclear import protein, is a likely candidate for trafficking the virus towards or through the NPC. TNPO3 is capable of directly binding HIV-1 integrase (IN), but the importance of this interaction to nuclear entry has been controversial [21,22,35], not least because dependence on TNPO3 has been genetically mapped to CA [18<sup>•</sup>,22,25<sup>•</sup>,26]. However, interactions between CA and Nup358 and CPSF6 could dictate the site of uncoating, either tethered to the NPC by Nup358 or in the cytoplasm, and thus whether integrase has the opportunity to encounter TNPO3. This possibility is supported by the behaviour of HIV-1 CA mutants. For example, the CPSF6 binding mutant CA N74D infects independently of Nup358 and TNPO3 and has retargeted integration preferences. Similarly, HIV-1 CA P90A infects independently of CypA and Nup358 and also retargets integration [3<sup>•</sup>,18<sup>•</sup>]. The nuclear basket component, Nup153, has also been suggested to bind integrase, again with dependence on Nup153 being mapped to CA [17,36]. More recently, Nup153 has been shown to interact directly with CA, binding the same pocket in CA which is bound by CPSF6 [48<sup>•</sup>].

#### CypA

It has been known for some time that cyclophilin A (CypA) plays a role in HIV-1 infection, with CypA binding to an exposed loop on the surface of the CA protein [37]. As a peptidyl-prolyl isomerase, CypA also catalyses *cis-trans* isomerisation of CA but, as for Nup358, an inability to separate binding and catalysis activities has hindered studies into whether isomerisation contributes to HIV-1 uncoating and infectivity [38,39]. Interaction between CA and CypA can be blocked through the use of cyclosporines or CA mutants G89V and P90A [40–42]. Despite a wealth of experimental data, understanding the role of CypA in infection has been difficult, not least because it varies between cell lines. New insight has come from the observation that blocking the interaction between CypA and CA relieves dependence on cofactors Nup358 and TNPO3, and subsequently changes integration site targeting [18<sup>•</sup>]. Importantly, cyclosporine (Cs) can be used to target CypA without inhibiting Nup358 Cyp recruitment. This has suggested that interaction between CypA and CA influences the course of HIV-1

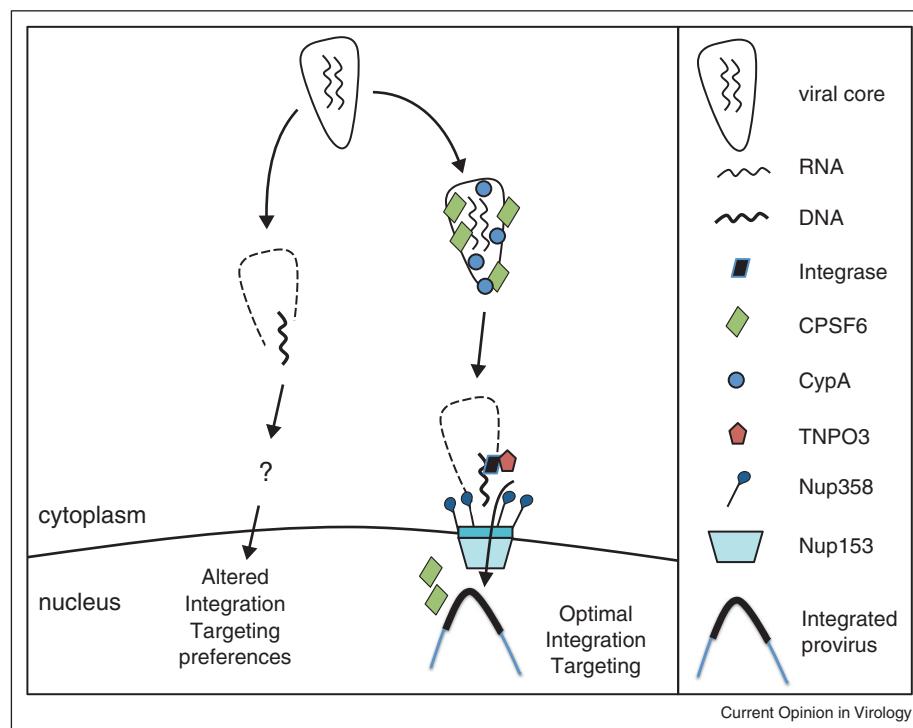
infection even in circumstances where its manipulation does not reduce infectivity. The mechanism by which CypA influences the route of nuclear entry remains intriguing but poorly understood.

### CPSF6

Cleavage and polyadenylation specificity factor-6 (CPSF6) was initially described as an HIV-1 inhibitory factor when a truncation of the murine variant was identified in a cDNA screen for restriction factors [3<sup>••</sup>]. CPSF6 is primarily nuclear, but manipulation of the C-terminal nuclear localisation signal results in its cytoplasmic accumulation and inhibition of HIV-1 infection [3<sup>••</sup>,32<sup>•</sup>,43<sup>••</sup>]. Inhibition depends on direct recruitment of CPSF6 by HIV-1 CA, and the co-crystal structure of a CPSF6 derived peptide bound to CA revealed details of the interaction [44<sup>••</sup>]. Single point mutations in either the truncated CPSF6 or CA are sufficient to ablate binding (F321N and N74D respectively) and rescue infectivity [3<sup>••</sup>,28,44<sup>••</sup>,45]. As mentioned above, the HIV-1 CA N74D CPSF6 binding mutant has been highly informative for understanding the role of CPSF6 as an HIV-1

cofactor. This mutant integrates with retargeted integration site preferences, essentially integrating into genes randomly [18<sup>••</sup>]. Importantly, this mutant also becomes insensitive to depletion of Nup358 and TNPO3 [3<sup>••</sup>] as do other CPSF6 CA binding mutants [44<sup>••</sup>]. We interpret these results as showing that CPSF6 directs HIV-1 into a particular pathway of nuclear entry that requires Nup358 and TNPO3 function. CPSF6 also appears to mediate CA's control of HIV-1 reverse transcription. The interaction between C-terminally truncated CPSF6 (delta NLS CPSF6) and the viral core delays both RT and uncoating [28,32<sup>•</sup>]. Whilst initial reports of the capacity of truncated CPSF6 to block RT have been conflicting [3<sup>••</sup>,28,45], a thorough investigation has determined that these discrepancies can be mapped to CPSF6 exon structure. CPSF6 mutants with a disrupted NLS but with the natural exon structure are capable of blocking viral DNA synthesis [43<sup>••</sup>,46<sup>••</sup>]. The mechanism of this inhibition remains to be clarified, but the data support a model in which CA recruitment of CPSF6 controls reverse transcription and therefore uncoating, as well as the subsequent recruitment of host cofactors. At present,

**Figure 1**



A hypothetical model of cofactor mediated HIV-1 nuclear entry and integration targeting. Shortly after entry into the cytoplasm CypA and CPSF6 are recruited to the viral core. These interactions suppress premature reverse transcription by a mechanism that remains unclear. CPSF6 recruitment allows HIV-1 to utilise the cofactors used by CPSF6 itself for nuclear entry, including TNPO3. At the NPC CA recruits the cyclophilin domain of Nup358. CPSF6 nuclear entry releases the virus enabling DNA synthesis. Nup358 use allows docking or tethering of the reverse transcription complex to the NPC, where appropriately orchestrated uncoating can expose the viral pre-integration complex for interaction with transport factors including TNPO3. In the absence of CypA or CPSF6 interaction, reverse transcription drives cytoplasmic uncoating, leading to Nup358 and TNPO3 independence and retargeted integration. We envisage a complex process of carefully orchestrated simultaneous reverse transcription, uncoating and integration events that have evolved to allow evasion of innate immune sensors. The use of proteins with a role in active transcription such as CPSF6, with its role in RNA 3' end processing, allows HIV-1 to target transcriptionally active chromatin.

one of the most confusing observations is the fact that, as for CypA, depletion or over expression of CPSF6 does not impact HIV-1 infection or replication in cell lines [3<sup>••</sup>,28,43<sup>••</sup>]. However, given that both CypA and CPSF6 appear to influence the route of HIV-1 nuclear entry, we propose that both of these proteins are important for HIV-1 even though they do not always impact infectivity when manipulated. This hypothesis is supported by the observation that neither the Cyp binding mutant HIV-1 CA P90A, or the CPSF6 binding mutant HIV-1 CA N74D, replicate in primary human macrophages [18<sup>••</sup>,47] and that manipulating CypA or CPSF6 interactions in these cells causes HIV-1 to trigger innate immune DNA sensors [46<sup>••</sup>].

## Conclusions

The mechanisms by which cofactors facilitate nuclear entry are still largely hypothetical and, like all good models, ours (Figure 1) is probably flawed, but testable. We note that the interpretation of data is complicated by the fact that the behaviours of NPC and nuclear transport proteins are likely to be interconnected. Thus manipulation of one, for example Nup358, has an impact on others, for example, TNPO3. It can therefore be challenging to establish whether a particular factor plays a direct role, or whether it impacts infection by regulating other members of a nuclear import pathway. However, it is reasonable to suppose that HIV-1 has evolved to make use of a connected series of proteins to optimally infect target cells and access preferred regions of the genome. We hypothesise that recruitment of CPSF6, a 3' end mRNA processing factor, is a way to target a pathway leading to the peripheral regions of chromatin containing the active genes that HIV-1 seeks. We imagine that defining the details of such a complex situation will require the collaboration of a variety of techniques including genetic, microscopic and biochemical approaches to eventually understand the molecular mysteries of lentiviral nuclear import. We propose that cell type specificity of cofactor use is important. Whilst HIV-1 infects several different cell types *in vivo* it seems unlikely that the same cofactors will be important for infection of distantly related cells, for example, activated T cells versus terminally differentiated macrophages. Thus it will be important to remember that negative data can be difficult to interpret, particularly if they appear to be cell type specific. We are confident that eventually our greater understanding of the processes of HIV-1 reverse transcription, uncoating and nuclear transport will be fundamental to our ability to manipulate infection both therapeutically and experimentally.

## Acknowledgements

We thank the members of the Towers lab, particularly Adam Fletcher, Jane Rasaiyah and Joanna Rowley for their insight into the models and ideas presented here. Our work is funded by Wellcome Trust Senior Fellowship no. 090940 to GJT, the National Institute of Health Research UCL/UCLH Biomedical Research Centre and the Medical Research Council.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Fassati A, Goff SP: **Characterization of intracellular reverse transcription complexes of human immunodeficiency virus type 1.** *J Virol* 2001, **75**:3626-3635.
  2. Miller MD, Farnet CM, Bushman FD: **Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition.** *J Virol* 1997, **71**:5382-5390.
  3. Lee K, Ambrose Z, Martin TD, Ozturk I, Mulky A, Julias JG, Vandegraaff N, Baumann JG, Wang R, Yuen W et al.: **Flexible use of nuclear import pathways by HIV-1.** *Cell Host Microbe* 2010, **7**:221-233.
  4. Arfi V, Lienard J, Nguyen XN, Berger G, Rigal D, Darlix JL, Cimarelli A: **Characterization of the behavior of functional viral genomes during the early steps of human immunodeficiency virus type 1 infection.** *J Virol* 2009, **83**:7524-7535.
  5. Arhel NJ, Souquere-Besse S, Munier S, Souque P, Guadagnini S, Rutherford S, Prevost MC, Allen TD, Charneau P: **HIV-1 DNA flap formation promotes uncoating of the pre-integration complex at the nuclear pore.** *EMBO J* 2007, **26**:3025-3037.
  6. Danielson CM, Hope TJ: **Imaging of HIV/host protein interactions.** *Curr Top Microbiol Immunol* 2009, **339**:103-123.
  7. Di Nunzio F, Danckaert A, Fricke T, Perez P, Fernandez J, Perret E, Roux P, Shorte S, Charneau P, Diaz-Griffero F et al.: **Human nucleoporins promote HIV-1 docking at the nuclear pore, nuclear import and integration.** *PLOS ONE* 2012, **7**:e46037.
  8. Lelek M, Di Nunzio F, Henriques R, Charneau P, Arhel N, Zimmer C: **Superresolution imaging of HIV in infected cells with FIAsh-PALM.** *Proc Natl Acad Sci U S A* 2012, **109**:8564-8569.
  9. Hulme AE, Perez O, Hope TJ: **Complementary assays reveal a relationship between HIV-1 uncoating and reverse transcription.** *Proc Natl Acad Sci U S A* 2011, **108**:9975-9980.
  10. Yang Y, Fricke T, Diaz-Griffero F: **Inhibition of reverse transcriptase activity increases stability of the HIV-1 core.** *J Virol* 2013, **87**:683-687.
  11. Forshey BM, von Schwedler U, Sundquist WI, Aiken C: **Formation of a human immunodeficiency virus type 1 core of optimal stability is crucial for viral replication.** *J Virol* 2002, **76**:5667-5677.
  12. Yang R, Shi J, Byeon IJ, Ahn J, Sheehan JH, Meiler J, Gronenborn AM, Aiken C: **Second-site suppressors of HIV-1 capsid mutations: restoration of intracellular activities without correction of intrinsic capsid stability defects.** *Retrovirology* 2012, **9**:30.
  13. Zhao G, Perilla JR, Yufenyuy EL, Meng X, Chen B, Ning J, Ahn J, Gronenborn AM, Schulten K, Aiken C et al.: **Mature HIV-1 capsid structure by cryo-electron microscopy and all-atom molecular dynamics.** *Nature* 2013, **497**:643-646.
  14. Dismuke DJ, Aiken C: **Evidence for a functional link between uncoating of the human immunodeficiency virus type 1 core and nuclear import of the viral preintegration complex.** *J Virol* 2006, **80**:3712-3720.
  15. Yamashita M, Emerman M: **Capsid is a dominant determinant of retrovirus infectivity in nondividing cells.** *J Virol* 2004, **78**:5670-5678.
  16. Yamashita M, Perez O, Hope TJ, Emerman M: **Evidence for direct involvement of the capsid protein in HIV infection of nondividing cells.** *PLoS Pathog* 2007, **3**:1502-1510.
  17. Matreyek KA, Engelman A: **The requirement for nucleoporin NUP153 during human immunodeficiency virus type 1 infection is determined by the viral capsid.** *J Virol* 2011, **85**:7818-7827.

18. Schaller T, Ocwieja KE, Rasaiyah J, Price AJ, Brady TL, Roth SL, •• Hue S, Fletcher AJ, Lee K, KewalRamani VN et al.: **HIV-1 capsid-cyclophilin interactions determine nuclear import pathway, integration targeting and replication efficiency.** *PLoS Pathog* 2011, **7**:e1002439.
- The first demonstration that capsid mutants that do not recruit CPSF6 and CypA integrate with different site preferences to wild type virus.
19. Brass AL, Dykxhoorn DM, Benita Y, Yan N, Engelman A, Xavier RJ, Lieberman J, Elledge SJ: **Identification of host proteins required for HIV infection through a functional genomic screen.** *Science* 2008, **319**:921-926.
20. Konig R, Zhou Y, Elleder D, Diamond TL, Bonamy GM, Irelan JT, Chiang CY, Tu BP, De Jesus PD, Lilley CE et al.: **Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication.** *Cell* 2008, **135**:49-60.
21. Christ F, Thys W, De Rijck J, Gijsbers R, Albanese A, Arosio D, Emiliani S, Rain JC, Benarous R, Cereseto A et al.: **Transportin-SR2 imports HIV into the nucleus.** *Curr Biol* 2008, **18**:1192-1202.
22. Krishnan L, Matreyek KA, Oztop I, Lee K, Tipper CH, Li X, Dar MJ, KewalRamani VN, Engelman A: **The requirement for cellular transportin 3 (TNPO3 or TRN-SR2) during infection maps to human immunodeficiency virus type 1 capsid and not integrase.** *J Virol* 2010, **84**:397-406.
23. Logue EC, Taylor KT, Goff PH, Landau NR: **The cargo-binding domain of transportin 3 is required for lentivirus nuclear import.** *J Virol* 2011, **85**:12950-12961.
24. Koh Y, Wu X, Ferris AL, Matreyek KA, Smith SJ, Lee K, KewalRamani VN, Hughes SH, Engelman A: **Differential effects of human immunodeficiency virus type 1 capsid and cellular factors nucleoporin 153 and LEDGF/p75 on the efficiency and specificity of viral DNA integration.** *J Virol* 2013, **87**:648-658.
25. Ocwieja KE, Brady TL, Ronen K, Huegel A, Roth SL, Schaller T, •• James LC, Towers GJ, Young JA, Chanda SK et al.: **HIV integration targeting: a pathway involving Transportin-3 and the nuclear pore protein RanBP2.** *PLoS Pathog* 2011, **7**:e1001313.
- The first demonstration that host factors Nup358 and TNPO3 have a role in targeting HIV-1 integration.
26. De Iaco A, Luban J: **Inhibition of HIV-1 infection by TNPO3 depletion is determined by capsid and detectable after viral cDNA enters the nucleus.** *Retrovirology* 2011, **8**:98.
27. Di Nunzio F, Fricke T, Miccio A, Valle-Casuso JC, Perez P, Souque P, Rizzi E, Severgnini M, Mavilio F, Charneau P et al.: **Nup153 and Nup98 bind the HIV-1 core and contribute to the early steps of HIV-1 replication.** *Virology* 2013, **440**:8-18.
28. Fricke T, Valle-Casuso JC, White TE, Brandariz-Nunez A, Bosche WJ, Reszka N, Gorelick R, Diaz-Griffero F: **The ability of TNPO3-depleted cells to inhibit HIV-1 infection requires CPSF6.** *Retrovirology* 2013, **10**:46.
29. Shah VB, Shi J, Hout DR, Oztop I, Krishnan L, Ahn J, Shotwell MS, Engelman A, Aiken C: **The host proteins transportin SR2/TNPO3 and cyclophilin A exert opposing effects on HIV-1 uncoating.** *J Virol* 2013, **87**:422-432.
30. Valle-Casuso JC, Di Nunzio F, Yang Y, Reszka N, Lienlaf M, Arhel N, Perez P, Brass AL, Diaz-Griffero F: **TNPO3 is required for HIV-1 replication after nuclear import but prior to integration and binds the HIV-1 core.** *J Virol* 2012, **86**:5931-5936.
31. Zhang R, Mehla R, Chauhan A: **Perturbation of host nuclear membrane component RanBP2 impairs the nuclear import of human immunodeficiency virus-1 preintegration complex (DNA).** *PLoS ONE* 2010, **5**:e15620.
32. De Iaco A, Santoni F, Vannier A, Guipponi M, Antonarakis S, •• Luban J: **TNPO3 protects HIV-1 replication from CPSF6-mediated capsid stabilization in the host cell cytoplasm.** *Retrovirology* 2013, **10**:20.
- An elegant demonstration that 2-LTR circle PCR can in certain cases detect autointegrants.
33. Tipper C, Sodroski J: **Enhanced autointegration in hyperstable simian immunodeficiency virus capsid mutants blocked after reverse transcription.** *J Virol* 2013, **87**:3628-3639.
- A nice SIV mutation study demonstrating that CA can control nuclear entry.
34. Bichel K, Price AJ, Schaller T, Towers GJ, Freund SM, James LC: **HIV-1 capsid undergoes coupled binding and isomerization by the nuclear pore protein NUP358.** *Retrovirology* 2013, **10**:81.
35. Cribier A, Segéral E, Delelis O, Parissi V, Simon A, Ruff M, Benarous R, Emiliani S: **Mutations affecting interaction of integrase with TNPO3 do not prevent HIV-1 cDNA nuclear import.** *Retrovirology* 2011, **8**:104.
36. Woodward CL, Prakobwanakit S, Mosessian S, Chow SA: **Integrase interacts with nucleoporin NUP153 to mediate the nuclear import of human immunodeficiency virus type 1.** *J Virol* 2009, **83**:6522-6533.
37. Gamble TR, Vajdos FF, Yoo S, Worthylake DK, Houseweart M, Sundquist WI, Hill CP: **Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid.** *Cell* 1996, **87**:1285-1294.
38. Bosco DA, Eisenmesser EZ, Pochapsky S, Sundquist WI, Kern D: **Catalysis of cis/trans isomerization in native HIV-1 capsid by human cyclophilin A.** *Proc Natl Acad Sci U S A* 2002, **99**:5247-5252.
39. Eisenmesser EZ, Millet O, Labeikovsky W, Korzhnev DM, Wolf-Watz M, Bosco DA, Skalicky JJ, Kay LE, Kern D: **Intrinsic dynamics of an enzyme underlies catalysis.** *Nature* 2005, **438**:117-121.
40. Braaten D, Franke EK, Luban J: **Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription.** *J Virol* 1996, **70**:3551-3560.
41. Franke EK, Luban J: **Inhibition of HIV-1 replication by cyclosporine A or related compounds correlates with the ability to disrupt the Gag-cyclophilin A interaction.** *Virology* 1996, **222**:279-282.
42. Luban J, Bossolt KL, Franke EK, Kalpana GV, Goff SP: **Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B.** *Cell* 1993, **73**:1067-1078.
43. Hori T, Takeuchi H, Saito H, Sakuma R, Inagaki Y, Yamaoka S: **•• carboxy-terminally truncated human CPSF6 lacking residues encoded by exon 6 inhibits HIV-1 cDNA synthesis and promotes capsid disassembly.** *J Virol* 2013, **87**:7726-7736.
- The first demonstration that CPSF6 recruitment has a role in controlling reverse transcription.
44. Price AJ, Fletcher AJ, Schaller T, Elliott T, Lee K, KewalRamani VN, •• Chin JW, Towers GJ, James LC: **CPSF6 defines a conserved capsid interface that modulates HIV-1 replication.** *PLoS Pathog* 2012, **8**:e1002896.
- The co-crystal structure of a CPSF6 derived peptide in complex with the N-terminal domain of HIV-1 CA convincingly demonstrated a conserved interaction and suggested a role in nuclear import.
45. Lee K, Mulky A, Yuen W, Martin TD, Meyerson NR, Choi L, Yu H, Sawyer SL, Kewalramani VN: **HIV-1 capsid-targeting domain of cleavage and polyadenylation specificity factor 6.** *J Virol* 2012, **86**:3851-3860.
46. Rasaiyah J, Tan CP, Fletcher AJ, Price AJ, Blondeau C, Hilditch L, •• Jacques DA, Selwood DL, James LC, Noursadeghi M et al.: **HIV-1 evades innate immune recognition through specific cofactor recruitment.** *Nature* 2013, **503**:402-405.
- This study suggests for the first time that HIV-1 cofactor use is linked to evasion of innate immune sensors.
47. Ambrose Z, Lee K, Ndjomou J, Xu H, Oztop I, Matous J, Takemura T, Unutmaz D, Engelman A, Hughes SH et al.: **Human immunodeficiency virus type 1 capsid mutation N74D alters cyclophilin A dependence and impairs macrophage infection.** *J Virol* 2012, **86**:4708-4714.
48. Matreyek KA, Yucel SS, Li X, Engelman A: **Nucleoporin NUP153 phenylalanine-glycine motifs engage a common binding pocket within the HIV-1 capsid protein to mediate lentiviral infectivity.** *PLoS Pathog* 2013, **9**:e1003693.
- This study demonstrates that Nup153 binds HIV-1 CA in the pocket bound by CPSF6 and PF74.