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Capsid–host interactions for HIV-1 ingress

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SUMMARY The HIV-1 capsid, composed of approximately 1,200 copies of the capsid protein, encases genomic RNA alongside viral nucleocapsid, reverse transcriptase, and integrase proteins. After cell entry, the capsid interacts with a myriad of host factors to traverse the cell cytoplasm, pass through the nuclear pore complex (NPC), and then traffic to chromosomal sites for viral DNA integration. Integration may very well require the dissolution of the capsid, but where and when this uncoating event occurs remains hotly debated. Based on size constraints, a long-prevailing view was that uncoating preceded nuclear transport, but recent research has indicated that the capsid may remain largely intact during nuclear import, with perhaps some structural remodeling required for NPC traversal. Completion of reverse transcription in the nucleus may further aid capsid uncoating. One canonical type of host factor, typified by CPSF6, leverages a Phe-Gly (FG) motif to bind capsid. Recent research has shown these peptides reside amid prion-like domains (PrLDs), which are stretches of protein sequence devoid of charged residues. Intermolecular PrLD interactions along the exterior of the capsid shell impart avid host factor binding for productive HIV-1 infection. Herein we overview capsid–host interactions implicated in HIV-1 ingress and discuss important research questions moving forward. Highlighting clinical relevance, the long-acting ultrapotent inhibitor lenacapavir, which engages the same capsid binding pocket as FG host factors, was recently approved to treat people living with HIV.

KEYWORDS HIV, capsid, trafficking, nuclear import, CPSF6, NUP153, FG domain, prion-like domain, mixed-charge domain, liquid-liquid phase separation, speckle-associated domain, lenacapavir

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INTRODUCTION

HIV/AIDS is a significant global health problem. At the same time, antiretroviral therapy (ART) has made a historical impact on the course of the HIV-1 pandemic. Prior to ART, HIV-1 infection in the vast majority of cases led to acquired immunodeficiency syndrome and death. Current ART regimens are typically composed of mixtures of antiviral compounds that target critical viral enzyme activities, including reverse transcriptase (RT) and integrase activities. Despite the huge positive impact of ART on public health, drug resistance is not uncommon, necessitating the development of new compounds that are active against drug resistant HIV-1 strains as well as new classes of drugs that target previously unexploited viral targets. Lenacapavir, which is a first-in-class capsid inhibitor, was recently approved to treat HIV-1 infection (1).

The capsid is a key substructure of the HIV-1 virus particle. HIV-1 particles assemble at the plasma membrane, through which the fledgling viruses bud (2). The resulting lipid-enveloped virions are roughly spherical, ~90–120 nm in diameter. Viral structural proteins [matrix (MA), nucleocapsid (NC), and capsid protein (CA)] and replication enzymes (protease, RT, and integrase) are produced during virus particle maturation via proteolytic cleavage of precursor Gag and Gag-Pol polyproteins, respectively (3). MA forms a spherical shell that interacts with the viral membrane (4, 5). Interior to the MA lattice lies the viral core. Roughly cone-shaped, the core is composed of the capsid outer shell, which itself is composed of CA. Core luminal components, which include two copies of the plus-stranded viral RNA genome, NC, RT, and integrase (6–8) (Fig. 1A), play critical roles during the early steps of HIV-1 replication.

HIV-1 ingress begins with entry of the virus particle into a susceptible CD4-positive cell and terminates with formation of the provirus, which is an integrated DNA copy

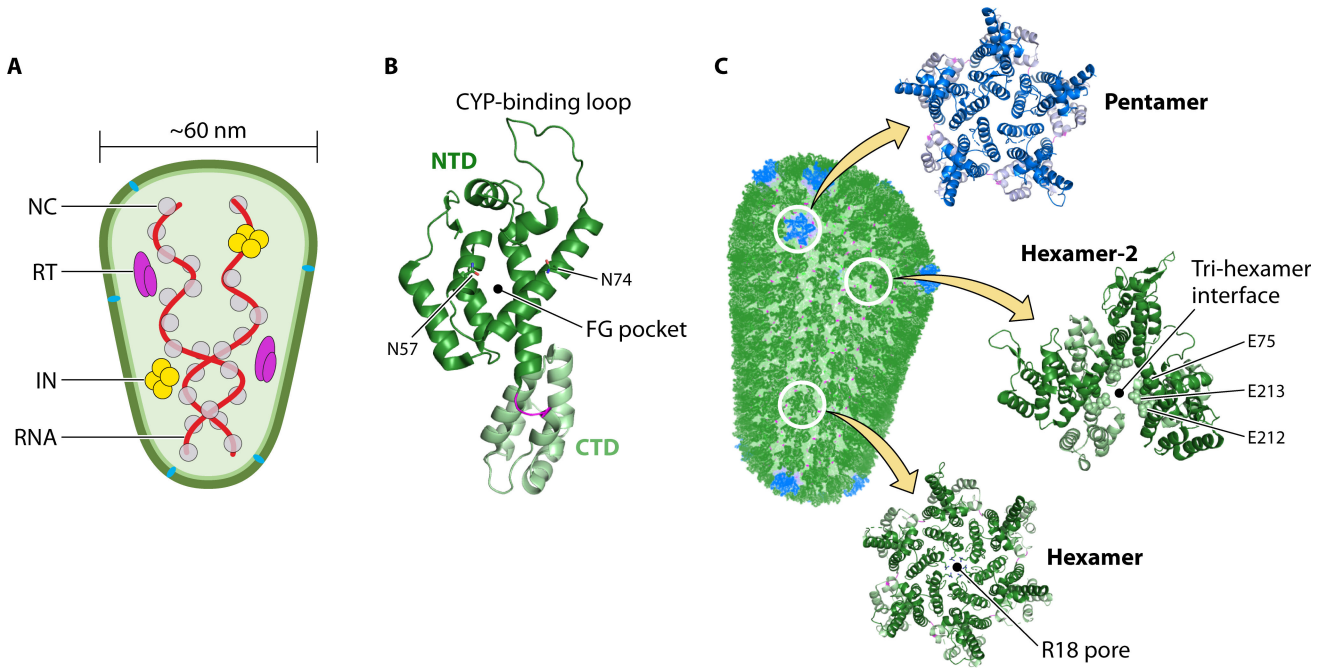

FIG 1 The HIV-1 core, CA, and capsomeres. (A) The drawing depicts the core with luminal components noted in text. IN, integrase. The approximate width of the wide end of the core is indicated. (B) Structure of the CA monomer (based on Protein Data Bank (PDB) accession code 4XFY) (9). The NTD, short interdomain linker, and CTD are colored forest green, magenta, and pale green, respectively. CYP-binding loop and FG pocket locations within the NTD are noted. N57 and N74 sidechains, which form part of the FG pocket, are drawn as sticks. (C) All-atom model of the capsid shell built from 186 hexamers and 12 pentamers (PDB accession code 3J3Y) (10) alongside resected CA hexamer [PDB code 4U0D (11)], pentamer [PDB code 3P05 (12)], and hexamer-2 [PDB code 6ECO (13)] structures. Hexamer and hexamer-2 colorings are the same as in panel B; pentamer-specific NTDs and CTDs are colored navy and light blue, respectively. Arg18 sidechains within the hexamer, which surround the R18 pore label, are shown as sticks. Coalesced NTD Glu75 and CTD Glu212 and Glu 213 residues are shown in space-fill at the tri-hexamer interface, where a single copy of each residue is labeled.

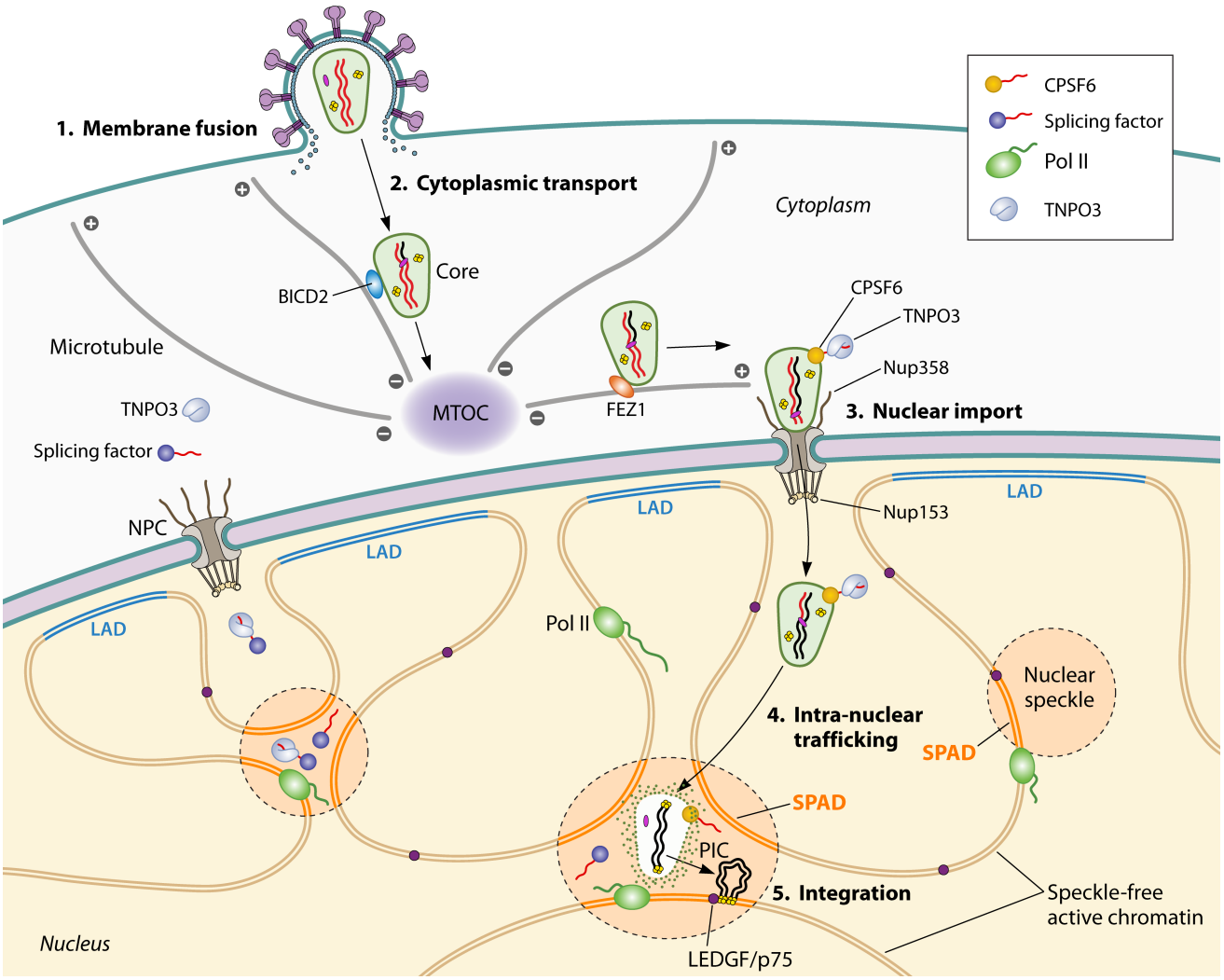

FIG 2 Schematic of HIV-1 ingress. Following virus-cell membrane fusion (step 1), the viral core in association with motor complex adapter proteins such as BICD2 and FEZ1 travels along microtubules (step 2) toward the microtubule organizing complex (MTOC)/nuclear membrane. The interaction of the core with cell proteins such as CPSF6, NUP358, and NUP153 facilitates transport through the nuclear pore complex (NPC) (step 3). The CA-CPSF6 interaction further licenses core incursion into the nucleoplasm (step 4) toward speckle-associated domain (SPAD) regions of chromatin for integration (step 5). The leftward flow depicts steady-state condition in the absence of HIV-1 infection whereby the β-karyopherin transportin 3 (TNPO3) engages pre-mRNA splicing factors in the cytoplasm to affect their nuclear transport and subsequent downstream targeting of nuclear speckles (43, 44). LAD, lamina-associated domain; LEDGF, lens epithelium-derived growth factor; PIC, preintegration complex; Pol II, RNA polymerase II.

of viral RNA (diagrammed as steps 1–5 in Fig. 2). While unintegrated HIV-1 DNA can support some level of viral gene expression (14–18), recent research has clarified that heterochromatinization restricts the transcriptional capacity of retroviral DNA prior to integration (19–24). Provirus formation, accordingly, critically determines productive HIV-1 replication (14–16, 25, 26). The early events of HIV-1 replication are dedicated to cell entry (Fig. 2, step 1: Membrane fusion), reverse transcription and trafficking of the viral replication machinery from the cell periphery to the nuclear membrane (step 2: Cytoplasmic transport), nuclear import (step 3), intranuclear trafficking to preferred sites of integration (step 4), and, finally, integrase-mediated integration (step 5). Research conducted over the past several years has highlighted a central role for the capsid in mediating the steps of HIV-1 ingress that occur after cell entry (Fig. 2, steps 2–5). At the same time, the capsid helps to regulate the detection of HIV-1 nucleic acids by intracellular innate immune sensing machineries (27–33). Herein, we overview details of

the capsid structure and common host factor binding elements within this structure. We then describe the myriad of cell factors that reportedly bind the capsid to aid its journey from the cell exterior to the nuclear interior. Along the way, we discuss potential experimental approaches to address lingering unknowns on the roles played by these host factors in HIV-1 ingress. We note several previous reviews that likewise discussed capsid–host interactions during the early steps of HIV-1 replication (34–42), some of which readers may wish to consult as background to the more-recently published works also presented here.

CAPSID STRUCTURE AND HOST FACTOR BINDING SITES

HIV-1 CA is an ~231-residue polypeptide that folds into two largely alpha-helical domains: the N-terminal domain (NTD) and C-terminal domain (CTD) (45, 46) (Fig. 1B). Independent CA molecules in turn interact intermolecularly to form distinct ring-like capsomeres, which are the building blocks of the capsid shell. The major capsomere is a hexamer of six CA molecules, while the minor capsomere is a CA pentamer (9, 12, 47, 48). The capsid shell is built from approximately 200 hexamers and exactly 12 pentamers (10, 49, 50) (Fig. 1C). The pentamers provide declinations to the otherwise regular hexameric honeycomb surface to induce curvature for shell closure. The distribution of seven pentamers at one end of the structure and five at the opposing end provides the cone shape typical of HIV-1 capsids (49).

Different types of *in vitro* assays are used by investigators to assess the binding of host factors to HIV-1 capsid, and it is worth noting that the nature of the binding assay can significantly influence the types of conclusions that can be drawn. Reactions performed with purified components, including proteins or peptides, can provide evidence for direct protein binding. Depending on reactants and techniques, affinity binding constants may be determined, and structure-based approaches such as NMR, X-ray crystallography, cryogenic electron microscopy (cryo-EM), or molecular dynamics (MD) may further yield atomistic models of bound complexes. Genetic-based approaches that then confirm the importance of the visualized protein-protein contacts in HIV-1 replication round out some of the most complete capsid–host interaction studies.

HIV-1 CA purified from recombinant sources such as *Escherichia coli* bacteria displays favorable biochemical properties, typically yielding protein concentrations as high as 20 mg/mL in isotonic salt-containing buffers. Early protein binding studies leveraged monomeric CA constructs including the isolated NTD (51–53). Determination of conditions that yielded the construction of stable CA hexamers (9, 47) subsequently led to their use in binding and structure-based studies, revealing novel contacts with the host that were missed from prior studies that utilized unassembled CA substrates (11, 54). Still, substrates that harbor the multi-hexamer honeycomb arrangement of the mature capsid lattice will capture interactions that exceed the binding capacities of individual CA molecules or capsomeres (Fig. 1C).

Recombinant CA-containing proteins under appropriate *in vitro* conditions template the formation of tube-like honeycomb arrays, a.k.a. capsid nanotubes, the surfaces of which mimic the surface of the mature capsid lattice (49, 55, 56). HIV-1 Gag is composed of MA-CA-spacer peptide 1 (SP1)-NC-SP2-p6 domains and recombinant CA-SP1-NC protein in the presence of templating nucleic acid formed nanotubes and capsid-like particles (CLPs) in isotonic salt conditions (49). These findings spearheaded the use of mature CA lattice-containing structures in host factor binding studies (53, 57–62). Because the comparatively large nanotubes/CLPs pellet by centrifugation at nominal *g*, host factor binding is oftentimes assessed via co-pelleting. Although CA on its own could also template nanotube formation (55), such structures required comparatively high salt concentration (1–2 M) to remain intact, limiting binding assays to nonphysiological salt conditions. Investigators have described two common workarounds of this bottleneck. One utilizes CA protein modified by cysteine replacement at positions 14 and 45 (A14C/E45C) (47), which, upon disulfide crosslinking, yields nanotubes that remain stable at isotonic salt conditions (63). The other advancement is inositol hexakisphosphate (IP6),

which, since its discovery as a CA pocket factor, has significantly widened physiologically relevant approaches to capsid-host factor interaction and structure-based studies. At pH 8, unmodified recombinant CA in the presence of IP6 can form nanotubes that remain stable in isotonic salt buffers. Assembly conditions at pH 6 moreover yield a predominance of CLPs (48, 56). Leveraging such CLPs in single particle cryo-EM studies (48, 64) in the coming years will predictably revolutionize the field to yield a wealth of information on the structural details of HIV-1 capsid-host interactions. Complementary advancements in structural methodologies include cryo-electron tomography to visualize host factors bound to native HIV-1 cores following perforation of viral membranes (65).

On the host factor side, binding is oftentimes measured in the context of cell extracts, which fails to address whether host-capsid interactions are direct or perhaps require unknown intermediators. In these latter cases, the detected binding is indirect. This importantly contrasts results of direct protein binding that can be gleaned from work

TABLE 1 CA-interacting host factors for HIV-1 ingress

Ligand	UniProtKB	Intracellular localization[a]	Binding	Binding site	Reference(s)
BICD2	Q8TD16	Cytosol	Direct	–	(66, 67)
CLASP2	A0A804HJG7	Cytosol	–	–	(68)
CLIP1	P30622	Cytosol; microtubule ends	–	–	(69)
CPSF6	Q16630	Nucleoplasm	Direct	FG pocket	(11, 54, 58, 70)
CYPA	P62937	Cytosol; nucleus[b]	Direct	CYP-binding loop	(51, 65, 71–73)
CYPB	P23284	Nucleus	Direct	CYP-binding loop	(71, 74)
DAXX	Q9UER7	Nucleus; nuclear bodies	–	–	(75)
DCTN1	Q14203	Actin filaments; cytosol	–	–	(76)
DIAPH1	O60610	Plasma membrane	–	–	(77)
DIAPH2	O60879	Nucleoli; ER	–	–	(77)
FEZ1	Q99689	Cytosol	Direct	R18 pore	(78, 79)
MAP1A	P78559	Cytosol	–	–	(80)
MAP1S	Q66K74	Cytosol	–	–	(80)
MAPK1	P28482	Cytosol; NS[c]	–	–	(81)
MARK2	Q7KZI7	Nucleoplasm; PM	–	–	(82)
MELK	Q14680	Cytosol; nucleus[d]	–	–	(83)
MX2	P20592	Nuclear membrane[e]	Direct	Tri-hexamer interface	(61, 62, 84–87)
NONO	Q15233	Nucleoplasm	Direct	HIV-2 CA residues 49, 101, 102	(33)
NUP153	P49790	Nuclear membrane	Direct	FG pocket; tri-hexamer interface	(11, 53, 54, 60, 88, 89)
NUP214	P35658	Nucleus	–	–	(59, 88, 90)
NUP358	H2QII6	Nuclear membrane; vesicles	Direct	CYP-binding loop	(91, 92)
NUP62	P37198	Nuclear membrane; vesicles	Direct	–	(88, 93, 94)
NUP88	Q99567	Nucleoplasm	–	–	(93)
NUP98	P52948	Nucleoplasm	–	–	(60)
PDZD8	Q8NEN9	Nucleoli; PM	–	–	(95)
PIN1	Q13526	Nucleoplasm; cytosol	–	–	(96)
PQBP1	O60828	NS	Direct	–	(31)
SEC24C	P53992	Vesicles	Direct	FG pocket	(90)
SUN1	O94901	Nuclear membrane	–	–	(97, 98)
SUN2	Q9UH99	Nuclear membrane	–	–	(97, 98)
TNPO1	Q92973	Nucleoplasm; cytosol	Direct	CYP-binding loop	(99)
TNPO3	Q9Y5L0	Vesicles	–	–	(100)
TRIM11	Q96F44	Nucleus; cytosol	Direct	–	(101)
TRIM34	Q9BYJ4	Nucleoli; centrosome	–	–	(102)
TRIM5α	Q9C035	Cytosol	Direct	Mature lattice	(103–105)

[a]Based on reference (106), unless noted otherwise. ER, endoplasmic reticulum; NS, nuclear speckles; PM, plasma membrane.
[b]From reference (107).
[c]Based on reference (108).
[d]From reference (109).
[e]From reference (110).

with purified components. Table 1 lists human proteins that have been reported to bind CA/capsid, most of which will be discussed in the main text, and whether these interactions have been shown to be direct. When known, the site/region of the capsid that mediates host binding is indicated. The table also lists the physical locations of the host factors within the cell. Following are descriptions of distinct aspects of HIV-1 CA and the capsid structure that serve as common host factor binding sites.

The CYP-binding loop

Cyclophilin (CYP) A and B, encoded by human *PPIA* and *PPIB* genes, respectively, were initially shown using the yeast two-hybrid assay to interact with HIV-1 Gag protein (71). CYPA was subsequently shown to directly bind an exposed loop within the CA NTD (the CYP-binding loop) (45, 51) (Fig. 1B). One consequence of the Gag-CYPA interaction is the incorporation of the host factor into virus particles (111, 112). However, subsequent research revealed that the interaction with the CYPA protein present in nascently infected target cells, as compared to the CYPA in virus particles, determines the host factor's role in HIV-1 infection (113, 114).

The CA-CYPA interaction can impact many steps throughout the early phase of the virus lifecycle, including cytoplasmic trafficking (107), nuclear import (115), and integration site targeting (52). Recent work has shown that CYPA can regulate the interaction of CA with other host factors, including cleavage and polyadenylation specificity factor 6 (CPSF6) (107), Sad1 and UNC84 domain containing (SUN) 1 (116), SUN2 (117), tripartite motif 5α (TRIM5α) (118, 119), and myxovirus resistance 2 (MX2) (120). Parallel studies have identified within the mature capsid lattice two CYPA binding sites in addition to the canonical CYP-binding loop (72, 73). Plausibly, the ability to regulate the interaction of several host factors with the capsid underlies the multifaceted role of CYPA in HIV-1 ingress.

Although much less studied than CYPA, the CA-CYPB interaction has also been shown to regulate HIV-1 nuclear import (74). All told, there are more than 20 human proteins with predicted cyclophilin-like domains, 7 of which can be depleted from cell extracts using glutathione *S*-transferase (GST)-tagged HIV-1 Gag in a pulldown assay format (121). The CTD of the large nucleoporin (NUP) NUP358 (a.k.a. RAN binding protein 2 or RANBP2), which is a component of the nuclear pore complex (NPC), contains a cyclophilin-homology (CypH) domain that engages the CYP-binding loop similarly as CYPA (52, 91, 92). Additional work is required to determine if cyclophilin domain-containing human proteins beyond CYPA, CYPB, and NUP358 play a role in HIV-1 ingress and, if so, where along the infection pathway they may exert their affects.

The Phe-Gly binding pocket

CPSF6 (11, 54, 70), NUP153 (11, 53, 54, 88), and SEC24 homolog C (SEC24C) (90) use specific Phe-Gly (FG) peptides to engage the FG-binding pocket (Fig. 3A). Although formulated primarily via the CA NTD (Fig. 1B), CTD elements from an adjacent CA molecule within the capsomere ring contribute important elements to the FG pocket (11, 54, 88, 90, 122). Recent research indicates that CA hexamers as compared to pentamers harbor functional FG-binding pockets (48, 123).

While CPSF6 contains but a single FG peptide, NUP153 and SEC24C contain 29 and 8, respectively. Most of these sequences are located within the C-terminal FG domain of NUP153 and the NTD of SEC24C (Fig. 3A). Remarkably, despite the comparatively high density of FG peptides within the NUP153 FG domain, capsid binding maps to a single FG sequence (53, 89). Recent work has shown that the key capsid-binding FG sequences within CPSF6, NUP153, and SEC24C reside within prion-like domains (PrLDs) (122) (Fig. 3A, yellow), which are low-complexity regions (LCRs) devoid of charged amino acid residues (124). PrLD sequences abutting the FG peptides moreover play critical roles in high-affinity capsid binding. Thus, while ~15 residue synthetic FG peptides bound recombinant CA hexamers comparatively weakly (K_d ~70 µM to 1 mM), the corresponding GST-tagged PrLD proteins bound purified HIV-1 cores with K_d's in the 0.3–0.9 µM

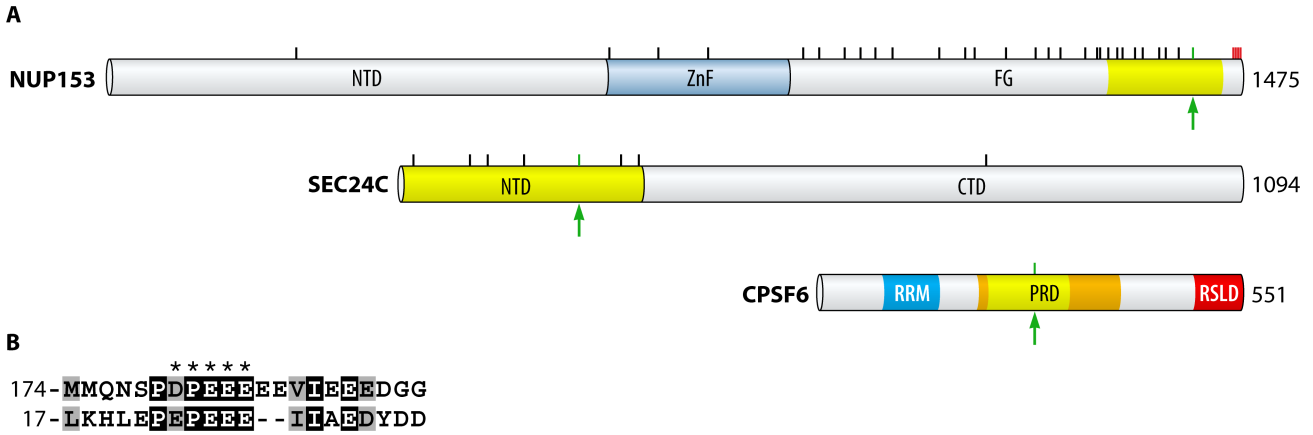

FIG 3 Host factor binding sequences and regions. (A) Domain arrangements of FG host factors NUP153, SEC24C, and CPSF6. Short vertical lines mark positions of FG peptides, with green arrows highlighting specific FGs for capsid binding. Yellow shade demarcates PrLDs as predicted from the PLAAC server (124). The red ticks at the NUP153 C-terminus demarcate the terminal RRRK$_{COOH}$ sequence (triple-Arg binding motif underlined). The CPSF6 PrLD lies amid a larger Pro-rich domain (PRD), which is denoted as orange rectangle. ZnF, zinc-finger domain; RRM, RNA recognition motif; RSLD, arginine-serine like domain [a.k.a. Arg-mixed charge domain or R-MCD (125)]. (B) Sequence alignment of similar stretches of FEZ1 (top) and PQBP1 (bottom) proteins. Numbers refer to amino acid positions within the respective proteins. Asterisks demarcate FEZ1 residues shown by MD to bind CA Arg18 at >87.8% occupancy, with E182 and E183 showing near complete occupancy (>99.5%). Black and gray backgrounds indicate identical residues and physiochemically conserved side chains, respectively.

range. The structural basis of the CPSF6 PrLD-capsid interaction was visualized at the comparatively low resolution of ~7.9 Å using CA nanotubes and single-particle cryo-EM, which revealed that the FG-anchored PrLDs interlinked intermolecularly to form chains of CPSF6 molecules between adjoined rows of CA hexamers (122). These data suggest that a specific type of FG/PrLD-mediated higher-order interaction underlies the structural basis for avid FG host factor interactions with the HIV-1 capsid. Further details of the CPSF6-capsid interaction will be enumerated by studying larger CPSF6 substrates that incorporate the host factor's N-terminal RNA recognition motif (RRM) and/or C-terminal Arg-Ser-like domain (RSLD) (Fig. 3A) and/or by improving the resolution of the cryo-EM maps. Parallel structure-based work with the PrLD segments of NUP153 and SEC24C will reveal if these domains form similar higher-order linked chains as observed for CPSF6.

SEC24C was identified via a mass spectrometry (MS)-based screen for capsid binding host factors using recombinant CA nanotubes as bait (90). Interestingly, this screen identified several additional host factors with FG sequences amid predicted PrLDs. Some of these proteins, such as NUP62, NUP98, and NUP214, were previously shown to co-pellet with CA-SP1-NC (60, 88) or CA nanotubes (93) *in vitro*. Additional work is required to determine if PrLD-embedded FGs are responsible for these host-capsid interactions, as well as for the interactions detected for other FG-containing host factors uncovered in the CA nanotube binding screen (90). Plausibly, the FG pocket mediates the binding of a comparatively large array of host factors for HIV-1 ingress.

The R18 pore

A locally enriched electropositive pore composed of Arg18 residues at the center of hexameric and pentameric capsomeres provides a binding platform for electronegative cellular components including relatively small metabolites and proteins (Fig. 1C). The binding of dNTPs to the R18 pore can facilitate their transport into the core for reverse transcription (126, 127). IP6 is incorporated into virions via engaging CA residues Lys158 and Lys227 in the context of HIV-1 Gag. Following maturation, IP6 is coordinated by the guanidino sidechain groups of the R18 pore (56, 128–130). Recent research has highlighted that IP6 mediates capsid assembly via kinetically trapping pentamers into growing hexameric lattices (131). As previously mentioned, its addition to CA assembly reactions at pH 6 yields a predominance of CLPs. Adding IP6 to permeabilized virions

(132) or isolated cores (133) moreover dramatically stimulated the synthesis of double-stranded DNA in so-called endogenous reverse transcription assays, yielding DNA products that were competent for integration (132). These assays hold great promise to reconstitute various aspects of HIV-1 ingress under acutely controlled *in vitro* conditions.

The kinesin adapter protein fasciculation and elongation factor zeta 1 (FEZ1) binds capsid to effect HIV-1 ingress (78) (discussed in detail in the capsid-host interactions for cytoplasmic transport section). MD simulations revealed that FEZ1 leverages a stretch of electronegative residues, namely Asp180, Glu182, Glu183, and Glu184, to engage the capsid via the R18 pore (79). Polyglutamine binding protein 1 (PQBP1) engages HIV-1 capsid to effect innate immune sensing via cyclic GMP-AMP synthetase (cGAS) (31, 134). The N-terminal 46 residues of PQBP1, which, like FEZ1, is enriched in electronegative residues (Fig. 3B), interacted with capsids in cells more robustly than did the C-terminal fragment composed of residues 47–265 (31). Moreover, binding was effectively negated by the R18G amino acid substitution in CA. Additional structure-based work is required to determine if PQBP1 electronegative residues analogous to those in FEZ1 directly engage the R18 pore.

Restriction factors and the tri-hexamer interface

The main treatise of this paper is CA–host interactions that promote HIV-1 infection. Cells additionally harbor so-called restriction factors, which can thwart retroviral replication at distinct steps of their replication cycles [see references (135) and (136) for recent reviews]. Because some restriction factors, namely TRIM5α from rhesus macaques (137), DAXX (138), TRIM11 (101), TRIM34 (102), and MX2 (139, 140), can inhibit HIV-1 ingress via engaging the incoming capsid (57, 61, 62, 75, 84, 85), we, for completeness, include brief descriptions of these CA-binding protein functionalities.

TRIM5α forms a higher-order hexagonal lattice encasing the HIV-1 capsid (103, 104), the consequences of which accelerate uncoating and impede reverse transcription (57, 137). While TRIM11 also restricts reverse transcription via accelerating uncoating (101), DAXX reportedly impedes HIV-1 DNA synthesis via stabilizing incoming cores (75). MX2 restricts HIV-1 ingress a bit further down the infection pathway. While reverse transcription proceeds normally, MX2 restricts the accumulation of nuclear forms of HIV-1 DNA (unintegrated and integrated), indicating that MX2 primarily inhibits nuclear import or destabilizes post-import viral DNA (139–141).

MX2 is a multidomain protein composed of an N-terminal unstructured region, followed by GTPase and stalk domains with interspersed bundle signaling elements [reviewed in reference (142)]. While protein oligomerization (61, 62, 85) and the GTPase domain (86) contributed to capsid binding, binding is principally determined via an $_{11}$RRR$_{13}$ triplet of arginine residues that reside near the protein's N-terminus (87). MD simulations have shown that these Arg residues can interact with the electronegative side chain-enriched tri-hexamer interface that is formed via the juxtaposition of three neighboring CTDs from three distinct CA hexamers (13, 143) (Fig. 1C). Recent work has implicated a triplet of arginine residues near the C-terminus of Nup153—$_{1472}$RRR$_{1474}$ (Fig. 3A)—in capsid binding and HIV-1 nuclear import (89). MD simulations moreover indicated that this RRR motif—similar to the one in MX2—engages the tri-hexamer interface. Thus, while the main FG binding determinant of NUP153 engages individual CA hexamers, two additional protein elements—the PrLD and C-terminal RRR motif—potentially engage in higher-order interactions to impart high-affinity NUP153 binding to capsid. Additional research is required to determine the extents that the PrLD versus RRR motif contribute to avid NUP153-capsid interactions.

CAPSID-HOST FACTOR INTERACTIONS DURING HIV-1 INGRESS

Following are the descriptions of the roles purportedly played by CA-interacting proteins in capsid cytoplasmic trafficking, nuclear transport, and intranuclear trafficking to sites of viral DNA integration. A wide variety of approaches are used to assess the roles of host cell factors in HIV-1 replication. With the advent of RNA interference (RNAi) in 2001

(144), researchers could effectively deplete specific host factors from mammalian cells to assess their influences on virus infection and replication. The subsequent application of CRISPR-Cas9 has significantly expanded ways in which researchers can deplete specific mammalian cellular factors (145). The advent of complementary knockdown technologies moreover helps to alleviate concerns that may arise from technique-specific off-target effects. If the targeted factor is nonessential for cell viability, CRISPR-Cas9 can further be used to generate knockout cell lines. HIV-1 mutant viruses with changes in CA residues that are known to effect capsid-host interactions are also commonly used in the field. Because any given CA change might affect interactions with more than one cell factor (see preceding section), comprehensive studies often combine the study of CA mutant viruses with RNAi/CRISPR-Cas9 depletion strategies.

HIV-1 capsid uncoating

Because measures of capsid uncoating are commonly employed in HIV-1 ingress studies, we would be remiss without a short overview of this subject. We by no means intend this to be comprehensive—readers are directed elsewhere for reviews dedicated to HIV-1 uncoating (146–148).

Operationally, uncoating refers to loss of CA or capsomeres (or perhaps larger chunks) from the capsid shell during HIV-1 ingress. Both biochemical and microscopy-based approaches have been used to assess uncoating. Two intermediates of retroviral early event replication, namely, reverse transcription complexes (RTCs) (149, 150) and preintegration complexes (PICs) (151–153), are defined as high-molecular weight entities in cell extracts that support the processes of reverse transcription and integration, respectively. Initial analyses of partially purified HIV-1 RTCs and PICs by western immunoblotting revealed little, if any, CA content (150, 154, 155). Evidence for CA among active PIC fractions was subsequently revealed using more sensitive enzyme-linked immunosorbent assays and immunoprecipitation techniques (156). The fate-of-the-capsid (FOC) assay, which is a state-of-the-art biochemical uncoating assay, leverages the pelletability of CA soon after cell lysis. Intact capsids or other high molecular weight CA structures partition to the pelleted fraction while uncoated CAs (individual molecules or perhaps capsomeres) remain in supernatant fractions (57, 157). FOC assays are routinely used in studies that test the effects of specific host factors in uncoating. Advanced biochemical approaches that leverage fluorescent microscopy to monitor CA content of individual, permeabilized virions attached to glass slides have more recently been used to assess uncoating (158, 159).

The advent of advanced cell biology techniques (160) opened the door to *in situ* observation of replication intermediates in intact cells to assess HIV-1 uncoating. Numerous approaches, including accessibility of labeled RNA after virus-cell fusion (161) or loss of fluorescent marker proteins such as encapsulated, fluid phase green fluorescent protein (GFP) (162, 163), have been utilized. Systems that track loss or fluorescent fusion proteins with CA (164–166) or CYPA (167, 168) have also been used, though fluorescent tagging of important ingress factors such as CA or CYPA may subtly alter protein function. Recent attempts to mitigate potential off-targeting affects have leveraged the comparatively noninvasive labeling technique known as genetic codon expansion, where unnatural amino acid residues can be labeled *in situ* via click chemistry (169). Another confounding issue for ingress studies is that less than half of HIV-1 particles are infectious (170), necessarily complicating microscopy-based approaches. One ingenious workaround to this caveat is end-point dilution, whereby integration-dependent viral reporter genes are used to earmark cells productively infected via single viral particles (162, 168).

Different uncoating scenarios can be envisioned during HIV-1 ingress [comprehensively reviewed in reference (171)]. One posits that complete uncoating occurs soon after cell entry. While not uncommon in the early literature, the advent of microscopy-based approaches has since de-popularized this theory. Another posits progressive loss of CA during ingress, with perhaps nuclear transport requiring near-complete uncoating. This

stance was in part based on the long-held view that the width of the wide end of the HIV-1 core, ~60 nm (Fig. 1A) (50), exceeded the ~40–45 nm width of the NPC inner channel (172). However, recent work based on images from intact cells has indicated greater plasticity in NPC organization than once thought. In particular, the average inner diameter of 64 nm in T cells was sufficiently large to accommodate HIV-1 capsids for nuclear transport (173). These observations are consistent with other microscopy-based findings that HIV-1 capsids can remain largely intact during nuclear transport (163, 165, 169), with perhaps some remodeling required to pass through the nucleocytoplasmic sieve (166, 174, 175). Recent findings that nuclear factors such as CPSF6 and NUP153 (89, 122) display high affinity binding to mature capsid lattice substrates is consistent with the notion that some aspect of the mature lattice must survive nuclear transport and that HIV-1 uncoating primarily occurs post nuclear import (148, 163, 165, 169, 176, 177).

A key driving factor of HIV-1 uncoating is the physical process of reverse transcription (178–183). Indeed, imaging reconstituted endogenous reverse transcription reactions that also support integration has revealed fenestrations where apparent nascent DNA strands extrude from broken or cracked capsid shells (132). Such observations raise the intriguing possibility that integration may proceed before the capsid shell is fully disassembled.

Capsid-host interactions for cytoplasmic transport

The makeup of the intracellular environment, which is heavily populated by macromolecular complexes, precludes directional inward movement of large entities such as viruses or subviral complexes by passive diffusion [reviewed in reference (184)]. Viruses accordingly have evolved to hijack intracellular transport systems to navigate the cellular milieux. The cell harbors a cytoskeleton composed of actin microfilaments (~7 nm in diameter), intermediate filaments (~10 nm), and microtubules (~25 nm) [reviewed in reference (185)]. Microtubules are dynamic structures composed of α-tubulin and β-tubulin that enable the assisted trafficking of cargoes inward toward the nucleus (retrograde movement) or outward toward the cellular periphery (anterograde movement). The orientation of tubulin dimers within the polymers provides microtubules with intrinsic polarity. Microtubule plus-ends localize toward the cell periphery, while the minus-ends congregate with the microtubule organizing complex (MTOC), a comparatively large structure that, during interphase, often associates with the nuclear membrane (Fig. 2) [reviewed in reference (186)]. Different types of molecular motor proteins, namely dynein and kinesins, associate with microtubules to facilitate retrograde versus anterograde transport, respectively [see reference (187) for review].

Microtubules can become locally stabilized, which is associated with specific α-tubulin post-translational modifications including acetylation and detyrosination (188). HIV-1 infection induced the formation of stable microtubules, which required the function of microtubule plus-end tracking proteins (+TIPs) including microtubule-associated protein (MAP) RP/end-binding (EB) family member 1 (MAPRE1; a.k.a. EB1) (189), diaphanous-related formin 1 (DIAPH1), and DIAPH2 (77). While CA-SP1-NC nanotubes depleted DIAPH1 and DIAPH2 from cell extracts *in vitro*, MAPRE1 has not revealed capsid binding activity (69, 77). Interestingly, the sequence of the MAPRE1 C-terminal region mimics the sequence of the N-terminal portion of the HIV-1 CA CTD, which coincides with the phylogenetically conserved major homology region (69, 190). Thus, CA has seemingly evolved to mimic MAPRE1 to congregate +TIPs for effective microtubule engagement. Indeed, other +TIPs, namely cytoplasmic linker protein (CLIP)-associated protein 2 (CLASP2) (68) and CLIP1 (a.k.a. CLIP170) (69), have been shown to facilitate HIV-1 cytoplasmic transport and bind HIV-1 CA-SP1-NC nanotubes in cell extracts. MAPs MAP1A and MAP1S also facilitate microtubule stabilization and can interact with HIV-1 cores to affect their retrograde transport (80). Stabilized microtubules provide tracks for comparatively long-range motility, which is exploited by HIV-1 for the inward movement of its core.

HIV-1 capsids bind specific adapter proteins to engage ATP-metabolizing motors for microtubule-dependent transport. Eukaryotic cells harbor numerous kinesins but only one type of cytoplasmic dynein, which, during interphase, plays critical roles to traffic membrane-bound organelles, RNAs, protein complexes, and viruses [reviewed in reference (191)]. Dynein is a 13-component protein complex composed of heavy chains DYNC1H1 and DYNC2H1, intermediate chains DYNC1I1 and DYNC1I2, and various light intermediate and light chains. Dynein function requires the co-factor dynactin, which itself is a multi-component complex. Adapter proteins, which typically harbor coiled-coil domains, tether cargoes to the dynein-dynactin supracomplex to effect directional movement (191). The adapter protein bicaudal D homolog 2 (BICD2) enhanced the affinity of the dynein-dynactin interaction and dramatically activated the motility of the macromolecular complexes to move long distances *in vitro* (192, 193). BICD2 effectively bound CA/CA-SP1-NC nanotubes *in vitro* and was required for optimal HIV-1 infection and cytoplasmic trafficking (66, 67). By using purified recombinant BICD2 proteins, the interaction with capsid was moreover shown to result from direct binding (67). Several dynein/dynactin proteins in cell extracts also co-pelleted with CA-SP1-NC/CA nanotubes *in vitro*. Because the binding of these proteins to capsid lattices depended on the cellular concentration of BICD2, dynein/dynactin protein binding was almost certainly indirect and mediated via BICD2 (67) (Table 1). A separate study determined that the dynactin component DCTN1 can act as a +TIP to compete with CLIP1 for binding to HIV-1 cores and accordingly negatively regulate microtubule engagement and viral infection (76).

Kinesins comprise a comparatively large superfamily of ~45 related genes in mice that can be subclassified into 15 families [reviewed in reference (194)]. Based on the relative location of kinesin motor domains, family members can be broadly typed into N-kinesins (N-terminally located motors), M-kinesins (midregion located motors), and C-kinesins (C-terminal motors). While M-kinesins typically support retrograde transport of cargoes, N-kinesins typically support anterograde transport (194). Indeed, early studies implicated N-kinesins KIF4 (195, 196) and KIF3A (197) in Gag transport for retroviral assembly and release from cells. Perhaps counterintuitively, N-kinesins have also been implicated in retrograde transport of the HIV-1 core.

Depletion of cellular N-kinesin KIF5B was initially shown to delay the process of capsid uncoating in HIV-1-infected cells (198). Akin to dynein-dynactin, adapter proteins tether kinesin motor proteins to cargoes, and the kinesin adapter protein FEZ1 was subsequently shown to co-pellet with CA-SP1-NC nanotubes and facilitate the inward movement of HIV-1 cores (78). Purified FEZ1 proteins specifically interacted with CA hexamers and, as discussed above, MD simulations have yielded an atomistic model of electronegative FEZ1 residues that engage the R18 pore (79). Phosphorylation of FEZ1 by microtubule affinity-regulating kinase 2 (MARK2) in association with HIV-1 cores enhanced FEZ1's interaction with KIF5B and facilitated retrograde transport and HIV-1 infection (82). KIF5B moreover induced the relocalization of NUP358 from the nuclear membrane to the cytosol in a manner that was dependent on the capsid-CPSF6 interaction (199). Concordantly, capsid-CPSF6 complexes have been observed to traffic along microtubules (107). Plausibly, the connection of KIF5B with downstream factors such as NUP358 and CPSF6, which are predominantly associated HIV-1 nuclear import and intranuclear trafficking, respectively (199–203), instills overall retrograde movement for a kinesin that would usually move cargoes anterogradely. It also seems possible that KIF5B/FEZ1 could support the anterograde transport of HIV-1 cores from the MTOC to proximal NPCs for nuclear transport (Fig. 2) (199).

SEC24C is a member of the coat protein complex II or COPII complex that typically plays a role to transport cargoes from the endoplasmic reticulum to the Golgi apparatus (204), though a separate COPII component, SEC13, forms part of the NPC (205, 206). As described above, SEC24C was uncovered in an MS-based screen for host factors that interact with CA nanotubes (90). Knockout cells revealed a critical role for SEC24C in the production of HIV-1 under multi-cycle replication conditions. When analyzed under single round infection conditions, HIV-1 displayed similar 2- to 3-fold defects in core

stability, reverse transcription, and nuclear import, indicting an important role for this FG-pocket factor to maintain optimal core stability during HIV-1 ingress (90).

Phosphorylation can also reportedly regulate capsid stability during the early stage of HIV-1 infection. Maternal embryonic leucine zipper kinase (MELK) phosphorylates CA residue Ser149 for optimal reverse transcription and core uncoating (83). Peptidylprolyl *cis*/*trans* isomerase, NIMA-interacting 1 (PIN1) is a phosphopeptide-binding enzyme that isomerizes phosphorylated (S/T)-P peptide bonds (207). Virion-associated mitogen-activated protein kinase 1 (a.k.a. ERK2) phosphorylation of CA residue Ser16 was critical for the association of PIN1 with capsid and optimal core uncoating and HIV-1 infection (81, 96).

PDZ domain-containing protein 8 (PDZD8) was identified through a yeast 2-hybrid screen to interact with HIV-1 Gag and to play an important role in virus infection (208). PDZD8 in cell extracts was subsequently shown to co-pellet with CA-SP1-NC nanotubes, and RNAi-mediated knockdown reduced HIV-1 infection via accelerating the rate of capsid uncoating, which negatively impacted reverse transcription (95). Perhaps unexpectedly, HEK293T and HeLa cell clones knocked out for PDZD8 expression supported the wild-type levels of virus infection, indicating that PDZP8 is not an essential HIV-1 host cofactor (209).

The NPC and HIV-1 nuclear import

Nucleocytoplasmic transport is regulated by the NPC, a massive assembly of NUPs that precludes passive diffusion of comparatively large macromolecules (210). Eukaryotic cells have accordingly evolved specific chaperon-like nuclear transport receptor (NTR) proteins, also called β-karyopherins, to assist in the nucleocytoplasmic transport of comparatively large cellular cargos [reviewed in reference (211)]. Occasionally, cellular cargoes can access the NPC through direct binding of one of its constitutive components (212).

The human NPC is constructed from ~33 NUPs arranged in 8-fold rotational symmetry [reviewed in reference (206)]. Various NUP subcomplexes, namely cytoplasmic NUPs, the Y-complex, inner ring NUPs, pore membrane proteins, and nuclear basket NUPs, come together to form the NPC. Of the NUPs that have been shown to interact with CA (Table 1), NUP358, NUP214, and NUP88 are cytoplasmic filament NUPs, NUP62 and NUP98 are inner ring NUPs, and NUP153 is a nuclear basket NUP. Nine of the 33 human NUPs contain FG-repeat domains, including NUP214, NUP62, NUP98, and NUP153. The molecular sieving property of the NPC is determined by conglomeration of NUP FG-repeat domains within the inner NPC channel (206). It is important to note that capsid binding activity does not necessarily translate to a role for the NUP protein in HIV-1 nuclear import. For example, NUP214 function has been mapped to the comparatively late step of HIV-1 mRNA nuclear export (59).

The HIV-1 capsid has evolved specific interactions with NPC components to affect its nuclear transport. The interaction with NUP358 likely docks HIV-1 cores at the NPC (52, 59, 199). Although capsid binding predominantly maps to the RBD4-CypH didomain composed of the C-terminal CypH and adjacent Ran-binding domain IV (52, 59, 94), CA-SP1-NC in cell extracts co-pelleted a C-terminal truncation mutant lacking these domains about 40% as efficiently as full-length NUP358 (59). Plausibly, this residual binding activity accounts for CypH-independent NUP358 functionality under certain conditions of HIV-1 infection (213). Although not classified as possessing an FG-repeat domain, NUP358 does harbor 22 FG dipeptides, including 19 upstream of the C-terminal didomain. None of these, however, is predicted to lie within a PrLD. Additional work is required to map NUP358 determinants upstream of the RBD4-CypH didomain that contributes to capsid binding.

Binding reactions conducted with purified proteins have shown that NUP358 and NUP153 interact directly with CA (52, 53). HIV-1 nuclear import is moreover impeded in NUP358 or NUP153-depleted cells (52, 59, 200, 214). The β-karyopherin transportin 1 (TNPO1 or TRN-1) has also been shown to directly bind CA (99). While this study revealed

significant HIV-1 uncoating and nuclear transport defects in TNPO1-depleted cells, other studies reported no obvious HIV-1 infection defect in cells depleted for TNPO1 (93, 215). Additional work is accordingly warranted to investigate the contribution of the CA-TNPO1 interaction to HIV-1 nuclear import.

The β-karyopherin TNPO3 (a.k.a. TRN-SR2) has also been shown to play a significant role in HIV-1 nuclear import (216). Although first thought to be mediated via an interaction with integrase, subsequent work showed that the requirement for TNPO3 during HIV-1 infection mapped to CA and not integrase (217). Additional work has indicated that the connection between TNPO3 and CA is predominantly indirect and mediated via CPSF6 (218, 219). CPSF6, which is a member of the cleavage factor I mammalian (CFIm) complex (220), was first implicated in HIV-1 biology via the ability of a C-terminal truncation variant to potently restrict HIV-1 infection and nuclear import (58). Subsequent work revealed that TNPO3 is the β-karyopherin responsible for CPSF6 nuclear import (221, 222). In this way, TNPO3 depletion partially relocalized the normally nuclear sequestered CPSF6 to the cytosol, the consequences of which restricted HIV-1′s access to the nucleus (218, 219). Although CA-SP1-NC could deplete TNPO3 from cell extracts (100), this binding may very well be mediated via CPSF6. Additional work with CPSF6 knockout cells (223) and purified TNPO3 protein (221) could help to clarify if TNPO3 binds CA in a CPSF6-independent manner. Follow-up work has confirmed a marginal role for the TNPO3-integrase interaction in HIV-1 nuclear import (224, 225).

The role of CPSF6 in HIV-1 infection is highly context dependent, in general requiring spreading replication assays with primary cells to see substantial effects. For example, HEK293T knockout cells, if anything, supported increased levels of HIV-1 single-round infection compared with control cells (223), similar to responses observed using other transformed cell types depleted for CPSF6 expression via RNAi (58, 226). While RNAi-mediated knockdown in primary macrophages yielded an approximate 2.5-fold infection defect under single round conditions (203), this approach in a separate study counteracted the ability for the cells to support spreading HIV-1 replication (29). CRISPR-Cas9-mediated depletion of CPSF6 from primary, resting CD4+ T cells moreover reduced HIV-1 infection ~7- to 10-fold under single round conditions (227). Approximate 2- to 3-fold reductions in HIV-1 nuclear import have been reported under certain CPSF6 knockdown conditions (201, 203).

An ingenious approach to the study of HIV-1 nuclear import leveraged a drug inducible forced dimer construct of NUP62, called Nup62DG (176). By staggering the time of drug addition, these investigators were able to assess the time required post-infection to achieve 50% nuclear import, which ranged from about 3–4 h in CD4+ T cells and macrophages to about 7 h in HeLa cells. The resistance of the P90A CA mutant virus, which is defective for CYPA binding (111), to the effects of Nup62DG (176) suggests a potential cyclophilin component to NUP62 dependency during HIV-1 infection. Additional work with factor-specific knockdowns under baseline infection and drug-induced Nup62DG restricted conditions could potentially flush out such a dependency.

Recent *in vitro* work with DNA-origami mimics of the human NPC (called NuPODs for NUPs organized by DNA) suggests a potential model for HIV-1 nuclear import (94). The NuPOD scaffold afforded specific placement of NUP domains, or in the case of NUP62, the full-length protein, in regions representative of the NPC cytoplasmic face (NUP358 RBD4-CypH didomain), nuclear face (NUP153 FG domain), or in between (NUP62). The NUP153 FG domain revealed strong preference for binding to comparatively curved regions of the capsid lattice, including the narrow “tip end” of HIV-1 cores that feature five capsomere pentamers (Fig. 1C). Based on co-pelleting of purified proteins with CA nanotubes, the NUP153 FG-repeat domain bound capsids at higher affinity than did either the NUP358 RBD4-CypH didomain or full-length NUP62 protein. Accordingly, after NUP358-mediated docking at the NPC, an apparent binding affinity gradient from the cytoplasmic side of the NPC toward the nucleoplasm, combined with the affinity of the NUP153 FG domain for the highly curved tip, would naturally orient the core

for tip-first insertion and passage through the NPC. While the tip-first model is consistent with some tomographic images of HIV-1-infected cells (173), there are several caveats with the NuPOD system that deserve further testing. First, while the FG domain seemingly fully accounts for NUP153's capsid binding activity (53), elements upstream of the RBD4-CypH didomain, as mentioned, contribute to the NUP358-capsid interaction (59). Larger versions of NUP358 need to be tested to confirm if indeed NUP153 binds HIV-1 capsids with higher affinity than does (full-length) NUP358. Second, although CA-containing nanotubes readily interacted with NUP62 in cell extracts (88, 93) and with purified NUP62 protein (94), they failed to engage NUP62-engrated NuPODs. Moreover, engrafted NUP62 presented a barrier that HIV-1 cores could not effectively breach (94). Numerous FG NUPs, including NUP62, have the ability to form hydrogels (228), and it seems possible that multiple NuPOD-engrafted copies surpassed the local NUP62 concentration required for hydrogel formation. How then could the HIV-1 core breach cellular NPCs? In cells, NUP62 forms the channel NUP heterotrimer (CNT) complex with NUP54 and NUP58 partner proteins (229), so reconstituted CNTs should be tested in place of NUP62 to see if the physiological trimeric complex might present less of a barrier than did engrafted NUP62. Tests with relevant β-karyopherins, including TNPO1 and/or TNPO3 (± CPSF6), could also be performed, as the physiological roles of such NTRs is to ferry cargoes through the NPC. Finally, HIV-1 infection can induce the relocalization of nuclear-membrane bound NUP62 to the cytoplasm to associate with HIV-1 cores (176, 230), indicating that viral infection may help to reduce the barrier-forming properties inherent to the inner NPC channel. Additional experiments should be performed to test whether the other CNT proteins are also similarly relocalized in response to HIV-1 infection.

Nuclear trafficking and HIV-1 integration

An over-expression screen for nuclear envelop-associated proteins identified SUN1 and, to a lesser extent, SUN2, as potent inhibitors of HIV-1 infection. Sensitivity to SUN1 mapped to CA, and SUN1 and SUN2 in cell extracts co-pelleted with CA-SP1-NC nanotubes *in vitro*. While SUN2 knockout yielded marginal ~2-fold defects in HIV-1 infection, there was no apparent infection defect using SUN1 knockout cells (97). While restriction of infection by over-expression and CA-binding activities are reproducible across studies (98, 231), the physiological roles of SUN1 and SUN2 in HIV-1 infection await clarification. One RNAi-based study indicated that integration was significantly impaired in SUN1 knockdown cells, though this defect was not associated with a parallel defect in HIV-1 infection (116).

CA-binding proteins can significantly influence sites of HIV-1 integration. HIV-1 integration into human DNA occurs nonrandomly, with preferences observed at multiple levels that span from local nucleotide sequence to global chromatin architecture [see references (232) and (233) for recent reviews]. HIV-1 integration displays marked preferences for annotations associated with active chromatin including genes, transcriptional activity, gene-dense regions (234), activating epigenetic marks (235), speckle-associated domains (SPADs) (236), and topologically associating domains (TADs) (237). As a consequence, integration significantly negatively correlates with heterochromatic markers including repressive epigenetic marks (235) and lamina-associated domains (202, 238), which locate outward toward the nuclear periphery in association with lamin proteins (239) (Fig. 2 and 4).

Gene knockout and RNAi studies have highlighted two virus-host interactions that in large part account for HIV-1 integration targeting preferences. One of these interactions occurs between integrase and chromatin-associated host protein lens epithelium-derived growth factor (LEDGF)/p75 (241–243). Although this manuscript centers on CA-interacting proteins, we will briefly touch upon the role of LEDGF/p75 in HIV-1 integration targeting. When LEDGF/p75 was depleted from cells, preferences for the above-mentioned active chromatin annotations decreased across the board, though at the same time each of these targeting metrics remained enriched relative to random

values (223, 242, 243). Under baseline infection conditions, HIV-1 integration favors gene mid-regions (244). In LEDGF/p75 knockout cells, this preference strikingly shifted to gene 5′ end regions, suggesting LEDGF/p75's role in HIV-1 integration targeting may be linked to transcriptional elongation (223, 245). Most recently, LEDGF/p75 has been shown to play an important role in the targeting of TADs (237).

Initial investigations into the roles of CA-binding host factors revealed comparatively minor effects in HIV-1 integration targeting. Thus, while NUP358 deletion revealed significant reductions in the targeting of gene dense regions, integration into genes was reduced only slightly (246). Similarly, NUP153 depletion in one study was reported to significantly reduce integration into gene-dense regions without affecting the frequency of intragenic integration targeting (247). A separate study however revealed significant 8% and 5% reductions in intragenic targeting frequencies upon knockdown of NUP153 and NUP98, respectively (60). Curiously, infections conducted in the presence of cyclosporin A, which is an inhibitor of the capsid-CYPA interaction (112), increased integration into gene-dense regions without significantly affecting the percentage of intragenic integration targeting (52).

The second virus-host interaction observed to play a dominant role in HIV-1 integration targeting is CA-CPSF6. In CPSF6 knockout cells, integration into genes was reduced by as much as 25%, similar to the effect observed in LEDGF/p75 knockout cells. However, in contrast to the results observed in LEDGF/p75 knockout cells, other chromatin targeting metrics, namely gene-dense regions, activating epigenetic marks, and SPADs, became disfavored when CPSF6 was knocked out (223, 236). As a consequence, heterochromatin marks, such as repressive histone modifications and LADs, can become favored integration targets in CPSF6 knockout cells (202, 223). LEDGF/p75 and CPSF6 are lentiviral and primate lentiviral-specific host factors, respectively (58, 248–251), and the integration targeting profile of the γ-retrovirus Moloney murine leukemia virus was largely unchanged in LEDGF/p75 and CPSF6 knockout cells (223, 251). At the extent analyzed, proximity to SPADs and nuclear speckles (NS) is the strongest genomic predictors of HIV-1 integration targeting (252).

Recent image-based work has drawn interesting parallels between intranuclear localization of HIV-1 cores and integration targeting preferences. Viruses unable to interact with CPSF6 (through CA mutagenesis or CPSF6 knockdown/knockout) were arrested for nuclear penetration and uncharacteristically targeted LADs for integration (165, 168, 201–203) (Fig. 4A). Initial reports of roles for NUPs and CYPA in HIV-1 integration targeting may be interpreted in light of these more recent findings. NUP358 or NUP153 depletion may similarly restrict the extent of nuclear incursion of HIV-1 cores, leading to significant decreases in integration in gene-dense regions, which strongly track with SPADs (252). Conversely, because CYPA binding regulates the CA-CPSF6 interaction (107), loss of CYPA may simply increase CPSF6 occupancy, translating to further penetration distance than observed under baseline infection conditions. Such hypotheses can be tested by quantifying intranuclear penetration distances of HIV-1 cores under NUP and CYPA knockdown conditions.

Under baseline infection conditions, CPSF6, independent of cell type, relocalized from broadly pan-nuclear (nucleoli excluded) patterns to distinct puncta that co-localize with NS (175, 177, 203, 236, 253). This dramatic reorganization of nuclear CPSF6 strictly depended on its interaction with CA (177, 203, 236). Consistent with the notion that reverse transcription terminates in cell nuclei (176, 177, 236), viral DNA synthesis can occur in the context of CPSF6-HIV puncta (253). Imaging of live cells has indicated that fluorescently labeled CPSF6-HIV puncta are long-lived and highly dynamic (236, 240). Recovery of fluorescently labeled CPSF6 puncta within ~2 to 4 min of photobleaching was interpreted as evidence for liquid-liquid phase separation (LLPS) (240). LLPS of macromolecules drives intermolecular interactions that underlie the formation of biomolecular condensates and cellular bodies, including NS (254–256).

These observations have suggested the following model for the role of CPSF6 in HIV-1 nuclear trafficking and integration targeting (Fig. 4B). CPSF6 displaces the capsid from

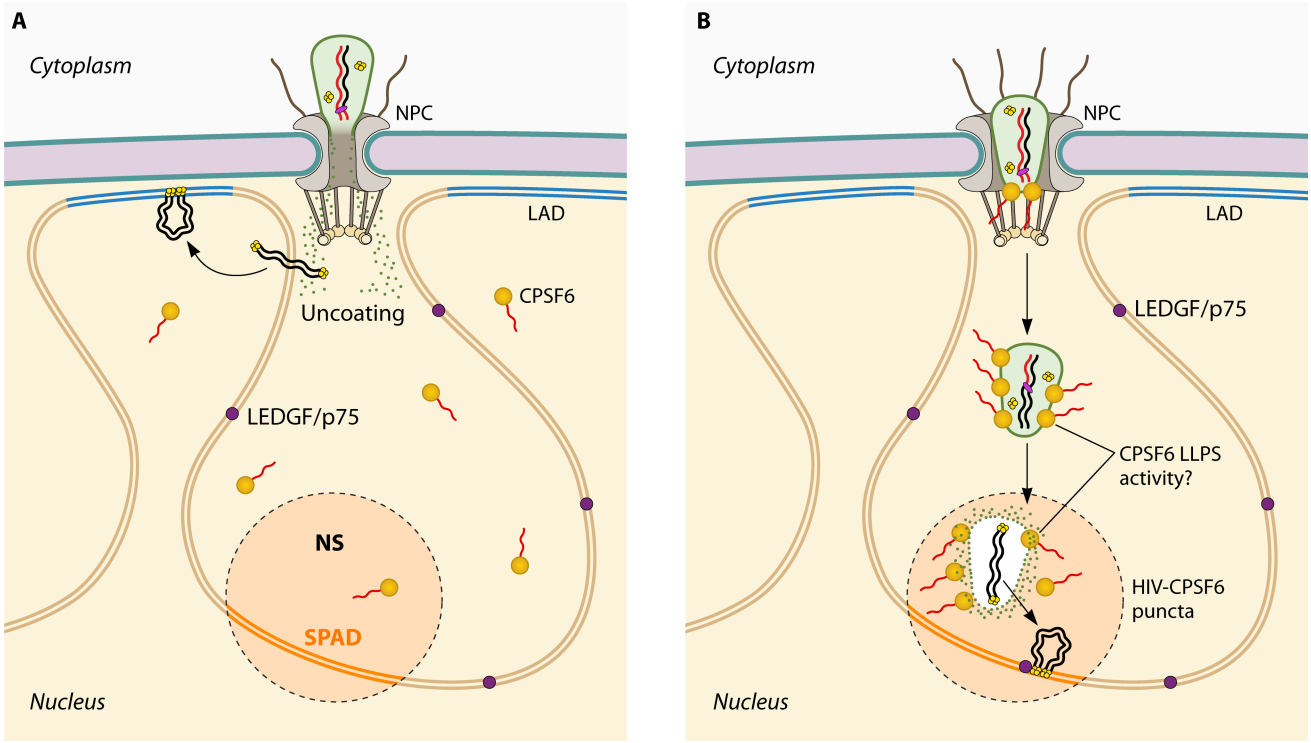

FIG 4 Roles for the CA-CPSF6 interaction in HIV-1 intranuclear trafficking and integration targeting. (A) In the absence of its interaction with CA, CPSF6 localization remains largely pan-nuclear, HIV-1 trafficking arrests at the nuclear pore complex (NPC), and integration occurs at proximal chromosomal locations including lamina-associated domains (LADs). (B) The CA-CPSF6 interaction is critical for nuclear evasion of the HIV-1 core, CPSF6 puncta formation, and SPAD-integration targeting. Recent work has raised the intriguing possibility that CPSF6 LLPS activity underlies HIV-induced puncta formation (240), which will require additional experiments to formally prove or disprove. NS, nuclear speckles; SPAD, speckle-associated domain.

its interaction with NUP153 at the nuclear basket to license core penetration into the nucleoplasm (203). CPSF6 LLPS activity may then help to determine HIV-CPSF6 puncta formation and integration into SPAD chromosomal regions (240, 257), though this has yet to be formally demonstrated. Consistent with a potential role for LLPS activity, fluorescently tagged CPSF6 RSLD fusion proteins co-localize with NS in cells and display LLPS activity *in vitro* (125). Moreover, hyperosmotic stress can induce the formation of cellular CPSF6 foci, which is indicative of LLPS activity (258), and recombinant mCherry-CPSF6 fusion protein displayed LLPS activity *in vitro* (259). Several questions immediately arise from these observations. For example, might CPSF6 LLPS activty primarily underlie visible punta formation at NS, or perhaps also play a role to translocate the HIV-1 core through the nucleoplasm? CPSF6 contains two separate LCRs (the PrLD and RSLD) and although each LCR on its own can pase separate *in vitro* (125, 259), the contribution of each region to CPSF6's overall LLPS activity needs to be established. Such observations raise the intriguing possibility that CPSF6 functionality beyond CA-binding might contribute to HIV-1 intranuclear trafficking and integration targeting. Additional questions include whether other capsid and/or CA-binding proteins play potential roles in CPSF6 puncta formation and HIV-1 integration targeting? Moving forward, we would propose that assays for CPSF6 puncta formation and SPAD-proximal integration should be incorporated into studies that examine the roles of CA-binding proteins in intranuclear trafficking and HIV-1 integration targeting.

CONCLUSIONS AND PERSPECTIVES

The HIV-1 ingress field has uncovered a dizzying array of human proteins that can interact with CA and the capsid (Table 1). Several questions arise based on these

observations. What fraction of the interactions that play demonstrative roles in growth promotion are required for HIV-1 to successfully infect any given cell? Plausibly, some of these interactions may be cell-type specific, while others may be equally important regardless of cell type. Given the applicability of CRISPR-Cas9 with primary cells associated with HIV-1 research (260, 261), we would highlight the inclusion of knockdown/knockout experiments with primary cells moving forward. Because each capsid is constructed from ~200 (or so) hexamers and exactly 12 pentamers (Fig. 1C), there are several binding sites available at any given point during HIV-1 ingress. Thus, it is theoretically possible (and seems probable) that interactions with multiple host factors could occur at any given point in time. It would be informative if future studies could extend analyses of new capsid-host interactions to test importance alongside factor(s) that were previously established to play an important role at the indicated point of the infection cycle.

As cytoplasmic trafficking transits to nuclear translocation, new sets of CA-host interactions will occur. How cytoplasmic-specific interactions transfer to interactions specific to nuclear import is largely a black box and should be one focus of future work. For example, is it important for NUP358 to displace CYPA from the CYP-binding loop, or does NUP358 engage unoccupied CYP-binding loops to dock the core at the NPC? Similarly, does NUP153 need to displace SEC24C from FG-binding pockets, or might NUP153 and SEC24 simultaneously bind the capsid to form higher-order PrLD interactions along the mature lattice? There is reasonable evidence to suggest that CPSF6 displacement of NUP153 from capsid is important to transition from nuclear import to intranuclear trafficking (203). In the preceding section, we have highlighted several interesting questions that arise from the potential involvement of CPSF6 LLPS activity to intranuclear trafficking and HIV-1 integration targeting.

One of the most important aspects of basic scientific research is the potential to inform translational medicines. LEN is a highly potent capsid inhibitor that displays greater potency to inhibit HIV-1 ingress as compared to the late steps of virus replication (262). Based on efficient competition with FG host factors for capsid binding (262, 263), it has been proposed that inhibition of capsid-host interactions in part underlies LEN's potency (263). However, the results of a more recent study have argued that inhibition of capsid-host interactions plays little if any role in determining the compound's potency (122). Drug binding at the interface of two separate molecules, in this case two adjacent CA molecules that compose a single FG pocket within the hexamer, can elicit allosteric affects that may alter structure/function distal from the point of drug binding, in this case decreasing capsid pliability that may be required for uncoating and integration (122). This story is reminiscent of the allosteric integrase inhibitors (ALLINIs) that bind HIV-1 integrase at the LEDGF/p75 binding site, which is likewise composed of two separate viral protein molecules (264, 265). Inhibition of integrase-LEDGF/p75 binding was initially thought to underlie ALLINI potency (265). However, follow-up work showed that these compounds elicit uncontrolled integrase polymerization (266, 267), the consequences of which primarily inhibit HIV-1 particle morphogenesis in a LEDGF/p75-independent manner (268). Thus, for both LEN and ALLINIs, due to the engagement of viral pockets that are composed of more than one target molecule, antiviral potency is driven by allosteric affects felt throughout higher-order structures: the mature capsid lattice in one case, and aberrant linear and branched chain integrase polymers in another. These highly unpredictable antiviral mechanisms of action should inspire investigators to seek small molecule inhibitors of other virus-host interactions, especially in cases where more than one viral molecule composes the complete host factor binding site.

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