Viruses in the Nucleus

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Viral infection is intrinsically linked to the capacity of the virus to generate progeny. Many DNA and some RNA viruses need to access the nuclear machinery and therefore transverse the nuclear envelope barrier through the nuclear pore complex. Viral genomes then become chromatinized either in their episomal form or upon integration into the host genome. Interactions with host DNA, transcription factors or nuclear bodies mediate their replication. Often interfering with nuclear functions, viruses use nuclear architecture to ensure persistent infections. Discovering these multiple modes of replication and persistence served in unraveling many important nuclear processes, such as nuclear trafficking, transcription, and splicing. Here, by using examples of DNA and RNA viral families, we portray the nucleus with the virus inside.

Viruses have long been studied not only as causative agents of human diseases, but also as extraordinary tools to explore cell biology, and in particular that of the nucleus. In fact, many elements of the eukaryotic transcription machinery have been elucidated with viruses. The first transcriptional promoter and enhancer elements were described in the simian vacuolating virus 40 (SV40) genome, a closed circular and superhelical polyomavirus discovered in 1960, found in monkeys and humans (Liu 2014). SV40 served to describe the binding of transcription factors (TFs) to the promoter, like SP-1 (Dynan and Tjian 1983), or the enhancer element (activator protein [AP]-1 and AP-2) (Lee et al. 1987). Together with adenovirus (AdV) major late promoter, SV40 served as an important in vitro experimental system for the discovery of basal and accessory TFs. Major discoveries of mRNA processing were made possible due to adenoviral infections (Berget et al. 1977; Chow et al. 1977), while polyadenylation was first observed with poxviruses. Apart from being fundamental for the discovery of the enzyme reverse transcriptase (RT) (Baltimore 1970; Mizutani et al. 1970) and for the initiation of recombinant DNA technologies, retroviruses together with DNA tumor viruses provide major contributions to our understanding of the origins of human cancers.

Viral genomes can assume different configurations: single- and double-stranded DNA (dsDNA), plus- or minus-stranded RNA, or double-stranded RNA. Retroviruses contain a positive-strand RNA molecule retrotranscribed into DNA, and as most dsDNA viruses (polyomaviruses, adenoviruses, herpesviruses, papillomaviruses [PVs]) they replicate in the nucleus.

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Among RNA viruses, only some negative-strand RNA viruses like influenza have a nuclear phase in their life cycle. Some of them require host cell polymerases to replicate their genome, while others, like adenoviruses and herpesviruses, encode their own replication factors. In both cases, viral replication is highly dependent on the cellular state and cellular permissiveness to replication. Among the viruses that will be explored here are several families of DNA viruses, one example of an RNA virus (influenza), as well as different members of the Retroviridae family.

- Herpesviruses cause a myriad of clinical conditions, ranging from mild skin sores to cancer. They have a large nonsegmented linear dsDNA genome, encoding for 70–200 different genes, and one of their best-known features is their capacity to establish latent infections. Here we discuss herpes simplex viruses (HSV)-1 and -2, Epstein–Barr virus (EBV), Kaposi's sarcoma–associated herpesvirus (KSHV) and cytomegalovirus (CMV).

- Adenoviruses are nonenveloped viruses first isolated from adenoids (tonsils). They contain a linear dsDNA genome, up to 48 kb, which enters the nucleus to replicate, relying entirely on host replication machinery. They mostly affect the respiratory tract and are subject to enormous investigations due to their delivery potential for gene therapy.

- PVs are nonenveloped dsDNA viruses causing skin warts or papillomas; some of them, like human papilloma virus (HPV) 16 and 18, can cause cancer. Upon infection, the viral genome replicates at low levels, resulting in escape from immune surveillance and ultimately cancer development.

- The hepatitis B virus (HBV) is a small virus of a peculiar structure and replication cycle. It contains a partially double-stranded and partially single-stranded circular DNA and its replication involves an RNA intermediate. It encodes its own polymerase, which has an RT activity, a DNA-dependent DNase activity, and an RNase activity. It causes hepatitis that is transmissible by blood and bodily fluids.

- Influenza viruses, causative agents of flu, contain a segmented negative strand viral RNA genome. The eight segments of RNA enter the nucleus in the form of viral ribonucleoproteins (vRNPs), containing viral proteins that possess nuclear localization signals.

- Retroviruses are a diverse family of RNA viruses encoding two copies of RNA genomes, which upon reverse transcription to viral DNA enter in the nucleus in the form of preintegration complex (PIC), can be rapidly loaded with histones, and are integrated into the host genome to ensure their replication. Types of retroviruses discussed here are human immunodeficiency virus 1 (HIV-1), human T-cell leukemia virus type 1 (HTLV-1), and murine leukemia virus (MLV).

NUCLEAR ENTRY AND NUCLEAR PORE COMPLEX

Entering the nucleus is one of the major challenges posed to a virus seeking the cellular machinery to transcribe and create progenies. Many viruses exploit the host cell nucleocytoplasmic trafficking mechanisms through the nuclear pore complex (NPC) to access nuclear functions, while others rely on the disruption of the nuclear envelope during cellular division. The mechanism of entry of viruses differs greatly and depends mainly on their size and on the properties of their viral proteins. Whereas some are small enough to passively diffuse through the NPC (e.g., HBV), others access the chromatin and integrate in the absence of this barrier (i.e., during mitosis, for example, PVs and MLVs, respectively), they can also attach to the NPC and from there release their viral genome (e.g., herpesvirus or adenovirus), or enter the nucleus via the NPC like lentiviruses (HIV-1) (Fig. 1).

The NPC is the largest macromolecular structure of the cell. Composed of ∼30 nucleoporins (Nups), the NPC is the gate for the entry/exit of molecules from the cytoplasm to the nuclei either by passive diffusion or by active transport (Beck and Hurt 2017). Intercalating the nuclear membrane, this structure forms cytoplasmic, inner, and nuclear rings, and peripherally extends both to the cytoplasm by
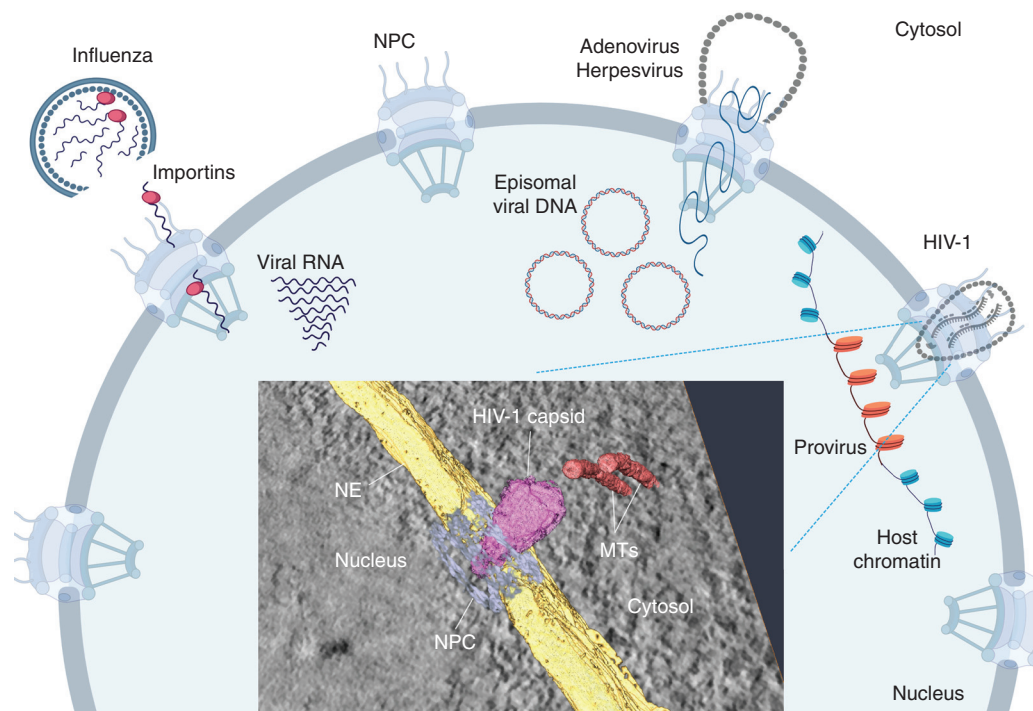
Figure 1. Viral nuclear entry. Influenza viral RNA enters the nuclei aided by importins while adeno-/herpesviruses attach to the nuclear pore complex (NPC) for the release of the genomic material. The lentivirus, HIV-1, travels with the capsid (CA). (*Inset*) 3D rendering of electron tomographic reconstruction showing the HIV-1 CA mutant A77 V (CPSF6-binding deficient) entering the NPC central channel in infected T lymphoblast SupT1-R5. (NE) Nuclear envelope, (MTs) microtubules. (Image kindly provided by Dr. Vojtech Zila and Erica Margiotta.)

cytoplasmic-oriented Nups and to the nucleus via nuclear basket Nups (Beck and Hurt 2017).

Nuclear entry of many viruses requires interaction with the NPC (Cohen et al. 2011; Fay and Panté 2015). This synergy was observed in HSV-1 infection, where electron microscopy (EM) showed the docking of capsid (CA) at the NPC (Batterson et al. 1983; Lycke et al. 1988). The viral DNA diffuses through the NPC, adopting a condensed rod-like structure (Shahin et al. 2006), after which empty CAs are released (Sodeik et al. 1997; Batterson et al. 1983; Lycke et al. 1988). CA binding to the NPC is maintained by two Nups, Nup214 and Nup358 (Copeland et al. 2009; Pasdeloup et al. 2009). On the other hand, several host response proteins, like MX2, might counteract this process (Huffman et al. 2017; Crameri et al. 2018). MX2 restriction goes beyond HSV-1, as it affects

the closely related viruses HSV-2 and KSHV (Crameri et al. 2018), as well as HIV-1 (Goujon et al. 2013; Kane et al. 2013), where it has been shown to interact with different Nups (Dicks et al. 2018; Kane et al. 2018).

NPC is undeniably one of the main structures of the cell whose functions viruses misappropriate to shut down immune responses and increase their replication. EBV hijacks the host nuclear entry complex by encoding for a kinase that phosphorylates Nup62 and Nup153, causing the redistribution of several Nups and favoring viral replication (Chang et al. 2015). Similarly, HIV-1 infection has been linked to Nup62 delocalization from the NPC to cytosol as it travels in association with vRNP during export via the Rev–vRNA RNP (Monette et al. 2011).

Adenoviral genome nuclear delivery is similar to that of herpesvirus, as CA is retained at

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the NPC. Linear dsDNA in the core of a CA shell associates with five viral proteins that aid the disassembly from the viral CA and docking to the NPC (Flatt and Greber 2017; Greber and Flatt 2019). The CA presents itself partially disassembled when docked at the NPC, a process mediated via Nup214 and Nup358 (Trotman et al. 2001; Strunze et al. 2011; Cassany et al. 2015). At the NPC, the recruitment of nuclear transport receptors supports the viral protein VII in the translocation of the viral DNA, which in many cases remains undelivered and accumulates in the cytosol in a CA-free form (Flatt and Greber 2017; Greber and Flatt 2019).

Influenza A virus (IAV) has a rigid shell, mainly constituted by M1 matrix protein, that protects the bundle of vRNAPs that are transformed into transcribed RNA in the nucleus. The shell dissociates in the cytoplasm and vRNAPs are transported into the nucleus via importins. Interestingly, histone deacetylase (HDAC)6 participates in the uncoating process, whereas nuclear import factor transportin 1 (TNPO1) promotes vRNAPs release. Freed vRNAPs can thus bind importins (α/β) for NPC translocation (Banerjee et al. 2014; Miyake et al. 2019).

The HIV-1 genome is translocated through the NPC into the nucleus in a form of a complex composed of the reversed transcribed viral DNA, CA, and integrase (IN) proteins. Reverse transcription and CA disassembly have long been considered to occur in the cytosol; however, a recent paper from Campbell's laboratory provides compelling evidence that this is completed in the nucleus. By using an inducible nuclear pore blockade system, they show that nuclear entry occurs with rapid kinetics that precedes the completion of reverse transcription (Dharan et al. 2020). The notion that PIC travels with intact CA structures through the NPC is consistent with recent reports of the presence of CA inside the nuclei of both T cells (Blanco-Rodriguez et al. 2020; Burdick et al. 2020) and macrophages (Bejarano et al. 2019). HIV-1 translocation into the nuclei depends on the protruding cytoplasmic Nup, Nup358, which binds CA through its cyclophilin (Cyp) domain (Schaller et al. 2011; Bichel et al. 2013). Nup153,

at the nucleoplasmic side of the NPC, has also been shown to be important for translocation through its direct interaction with CA (Matreyek et al. 2013; Buffone et al. 2018; Bejarano et al. 2019). However, it has remained poorly understood how the RT/PIC with an estimated diameter of ∼61 nm, composed of ∼1500 CA monomers organized in hexamers or pentamers, passes through the NPC, which has an inner diameter of ∼39 nm (Campbell and Hope 2015). A combination of 3D correlative fluorescence light and electron microscopy (CLEM) and cryo-electron tomography (CryoET) has now shed new light on the nuclear entry of HIV-1. The diameter of the NPC is sufficient to allow the nuclear import of intact, cone-shaped CAs, while the disruption of the hexagonal CA lattice occurs only once inside the nucleus (Fig. 1, inset; Zila et al. 2021).

HIV-1 nuclear entry is aided by numerous Nups interactions that hand-in-hand deliver the viral genomic content to the nucleus. The first visualization of the relationship between HIV-1 genomes and the NPC came in 2002 when HIV-1 RNA was shown to accumulate in close proximity or at the NPC (Cmarko et al. 2002). Inside the nucleus, HIV-1 viral DNA interacts with Nup153, Nup98, and the translocated promoter region (TPR), implicated in HIV-1 integration into the host genome (Di Nunzio et al. 2013; Lelek et al. 2015; Marini et al. 2015). The fact that passage through the NPC represents a functional bottleneck for HIV-1 replication is further supported by the presence of chromatin readers, splicing, and polyadenylation factors that aid HIV-1 integration. Particularly, cleavage and polyadenylation specificity factor 6 (CPSF6) and lens epithelium-derived growth factor (LEDGF/p75) are both tethered to the NPC via Nup153 and TPR, through binding of HIV-1 CA and IN, respectively (Lelek et al. 2015; Bejarano et al. 2019). Moreover, HIV-1 recurrently integrates in chromatin enriched for active transcription and super-enhancer (SE) markers (H3K4me1, H3K27ac, bromodomain-containing 4 [BRD4], and mediator of RNA polymerase II transcription subunit 1 [MED1]), clustering sites of integration >1 Mb apart (Lucic et al. 2019). Interestingly, develop-

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mentally regulated and tissue-specific genes have been shown to bind Nups and harbor enhancer markers, both in human, mouse, and *Drosophila* cells (Ibarra et al. 2016; Pascual-Garcia et al. 2017; Scholz et al. 2019).

INTERACTIONS WITH THE CELLULAR GENOME

Upon nuclear entry, the viral genome can either maintain an episomal form or integrate into the host genome. Either way, most viral genomes become highly chromatinized upon nuclear entry (i.e., gain nucleosomes, interact with TFs and chromatin modifiers, and present histone modifications contributing to their gene expression) (for review, see Knipe et al. 2013). Studies on the interaction between the viral and host genomes started evolving with the development of genome-wide capturing techniques, namely, chromosome conformation capture and its derivatives (3C, 4C, and later Hi-C) (for review, see Dekker et al. 2013; Denker and de Laat 2016).

One of the first viruses under scrutiny by these technologies was HIV-1. Initially, a loop between the proviral 5′ and 3′ long terminal repeats (LTRs) was linked to active viral transcription (Perkins et al. 2008). Subsequently, interactions between HIV-1 and chromosome 12 were observed using HIV-1 provirus as bait. Interestingly these interactions are lost upon activation of the lymphocytes and thus seem related to maintaining a state of repression (i.e., viral latency) (Dieudonné et al. 2009). More recently, a comprehensive Hi-C contact map from T Jurkat cells was cross-compared to HIV-1 insertion sites sequenced from patients and in vitro infected primary T cells. HIV-1 insertions occur mostly in transcriptionally active compartments (Chen et al. 2017) that display the highest contact frequency and strongly interact with each other (Lucic et al. 2019). Moreover, HIV-1 displays integration biases for cell-type-specific SEs (>30% of HIV-1 recurrent integration genes are in classified SEs in primary CD4$^+$ T cells), specifically for those in A1 active subcompartments (Fig. 2; Chen et al. 2017; Lucic et al. 2019). The importance of genome organization and an integration bias toward active compartments were

also proposed through a biophysical model of HIV-1 integration on chromatin fibers (Michieletto et al. 2019).

Most DNA viruses maintain their genomes extrachromosomaly in dividing cells, where their episomal genomes are tethered to cellular chromosomes by a virally encoded protein (Coursey and McBride 2019). Interactions with the host genome can result in disruption of cellular physiology and lead to cancer (EBV, HPV), DNA damage (parvovirus), or cause transcriptional aberrations (IAV).

The parvovirus, minute virus of mice (MVM), is a highly infectious rodent virus that causes DNA damage and uses S phase to begin replication. A 3C analysis directed at the viral genome (V3C) showed the interaction between MVM and the host DNA. These sites, termed virus-associated domains (VADs), correlate strongly with sites of DNA damage, occurring also in mock infected cells during the progression through the S phase of the cell cycle (Majumder et al. 2018). PVs establish persistent replication cycles, where upon initial infection the viral genome replicates with low levels of gene expression. Tethering of the viral and host genomes is enabled by the E2 PV protein, and in particular by the DNA-binding domain (DBD), which shows strong similarities with latency-associated nuclear antigen (LANA) and Epstein–Barr nuclear antigen (EBNA) proteins, responsible, respectively, for tethering of KSHV and EBV to cellular genomes (Coursey and McBride 2019). All three viral proteins bind to the transcriptionally active regions of chromatin, possibly as a result of their roles in viral transcription. However, chromosomal interactions between PV and EBV seem to differ, as PV interacts with transcriptionally active regions of the genome, EBV was found tethered to heterochromatic regions, as assessed by Hi-C (Moquin et al. 2018).

The transforming potential of EBV is in part linked to activation of MYC and other oncogenes via its own proteins. Upon infection of B lymphocytes, EBNA2, one of the six viral nuclear proteins, causes MYC up-regulation by promoting long-range enhancer and promoter looping (Zhao et al. 2011). This study paved the way to a more comprehensive understanding of
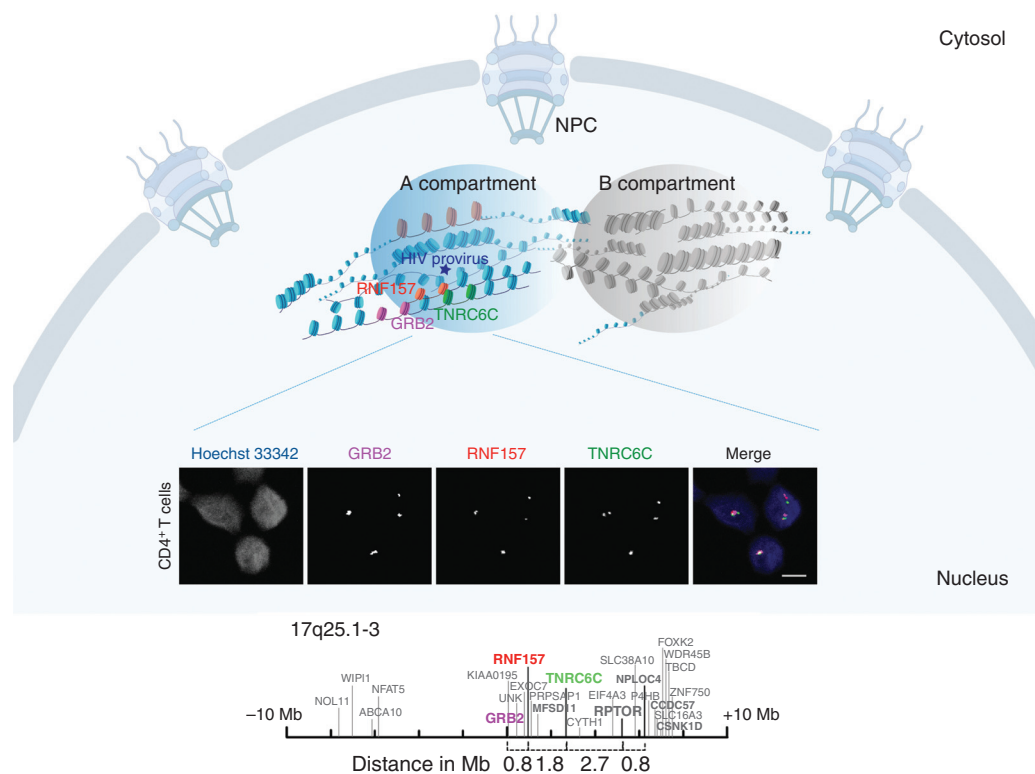
Figure 2. Integration of HIV-1 provirus favors active A compartments. (*Upper* panel) Cluster of genes recurrently targeted by HIV-1. Triple-color fluorescence in situ hybridization (FISH) of GRB2 (magenta), RNF157 (red), and TNRC6C (green) in resting and activated CD4$^+$ T cells. Maximum projections for each of the channels and merge images are shown. Nuclei were counterstained with Hoechst 33342 (blue). Scale bar, 4 μm. (*Lower* panel) Distribution of recurrent integration genes (RIGs) (bold) and single HIV-1 integration sites (plain) at the chromosomal region 17q25.1-3 within 20 Mb. Genes analyzed by triple-color FISH are depicted in magenta, red, and green. Distances between them on the linear DNA are indicated. (Images made by the Lusic laboratory in collaboration with V. Roukoss.)

EBV in cellular transformation. In EBV transformed lymphoblastoid cell lines (LCLs) 2000 enhancer sites are occupied by EBV proteins as well as by SE markers (Zhou et al. 2015), with 30% of genes important for LCL growth and survival representing EBV SEs (Jiang et al. 2017). Apart from EBNA2, other EBV proteins, like EBNA leader protein (EBNA-LP) and EBNA3C, were also linked to cellular transformation (Szymula et al. 2018), pointing to a concerted action of EBV proteins in taking over genome organization. Finally, tethering of EBV to the cellular genome seems to depend on EBNA1, as shown by 4C experiments in latently infected EBV cell lines. As this tethering persists

during mitosis as well, it is plausible that such interactions can be conserved in cycling cells (Kim et al. 2020).

HBV is transcribed in the nucleus from a viral covalently closed circular DNA (cccDNA) form (Newbold et al. 1995). Its interactions with the genome were assessed by a combination of Hi-C with viral DNA capture (CHi-C) (Moreau et al. 2018) in primary human hepatocytes, revealing that cccDNA interacts with positively marked CpG islands, occupied by RNA polymerase II (RNAPII), H3K4m3, H3K27ac, and CXXC-type zinc finger protein 1 (Cfp1) TF. Some of these CpG islands overlap with hepatocyte-specific genes and are deregulated upon

infection. A parallel study using 4C also linked cccDNA with actively transcribed regions, enriched for positive histone modifications and occupied by the HBV *trans*-activating protein HBV X (HBx) (Hensel et al. 2018).

The study by Moreau et al. also assessed chromatin interactions of the adenovirus type 5 (Ad5) in hepatocytes derived from an infected patient. The episomal Ad5 binding is skewed to regions enriched for active marks, similar to those of enhancers and transcription start sites (TSSs) and occupied by the forkhead box A (FOXA) TF (Moreau et al. 2018). Ad5 infection causes a striking global change of histone profiles, most of which is dependent on the first produced viral protein E1A (Horwitz et al. 2008). Viral protein VII complexes with nucleosomes, and its chromatin association is dependent on posttranslational modifications. Protein VII also directly binds high mobility group protein B1 (HMGB1), which remains attached to chromatin instead of being released as an inflammatory signal (Avgousti et al. 2016). This nucleosome binding is hence used by the virus as a strategy of immune invasion. Cellular transformation by adenovirus is initiated by viral protein E1A and its small isoform e1a, which causes a significant reshuffling of the active H3K18ac mark. By erasing almost 95% of the existing peaks and reestablishing new ones at promoter regions of genes involved in cell cycling, the virus appropriates cell propagation (Ferrari et al. 2012). E1A simultaneously binds histone acetyltransferase p300/CBP and the tumor suppressor retinoblastoma (RB) protein, which is locked into a repressing conformation upon p300 acetylation. This results in repression of genes that would otherwise be activated to inhibit viral replication (Ferrari et al. 2014).

IAV causes a strong transcriptional response of the host cell and induces changes in the nuclear morphology (Terrier et al. 2014). Of eight nonstructural (NS) proteins encoded by the virus, NS1 impacts genome organization and transcription of infected human macrophages. Changes in gene expression and 3D organization occur already at the early stages of infection, as revealed by Hi-C and RNA-seq. Many genomic regions transition from repressive hetero-

chromatic (B) to active, euchromatic (A) compartments, especially genes involved in type I/III interferon (IFN) pathways. This transition has been attributed to RNAPII readthrough transcription past the end of genes, mediated by NS1 protein. Dragging of RNAPII through the genome has been proposed to perturb CCCTC-binding factor (CTCF) sites and thus affects genome demarcation (Heinz et al. 2018). This is in agreement with the findings of Bauer et al., who used native elongating transcript sequencing (mNET-seq) in IAV infection and reported production of downstream-of-gene transcripts (DoGs). Contrary to the first study, the latter did not attribute RNAPII readthrough to NS1 (Bauer et al. 2018). However, it seems plausible that NS1 indeed plays a role in RNAPII readthrough, based on its capacity to bind and inhibit the function of CPSF complex, cleavage, and polyadenylation factor (Noah et al. 2003). A role for NS1 protein in RNAPII readthrough was further strengthened by the finding that NS1 partitions into nuclear ribonucleoprotein complexes, depending on the conjugation of the small ubiquitin-like modifier (SUMO) to the site embedded in a PDZ domain. NS1-SUMO virus caused a pervasive run-through into downstream genes due to interference with 3′ end cleavage and termination, which in turn causes splicing defects at later time points (Zhao et al. 2018). The evolution of domains in viral strains might thus confer different properties and advantages to viruses.

HTLV-1, a retrovirus capable of provoking aggressive malignancies in CD4$^+$ T cells in addition to the viral genes encoding for enzymes and structural proteins, contains regulatory and accessory genes (Bangham 2018). HBZ, one of the accessory genes required for clonal persistence of the integrated virus, is expressed from the minus strand, while plus strand expression necessary for viral propagation is suppressed in vivo. CTCF, a master regulator of chromatin structure and gene expression, binds to the proviral genome and acts as an enhancer blocker, causing long-distance looping between viral and host genome, thus resulting in changed patterns of gene expression (Satou et al. 2016; Melamed et al. 2018). Long-range interactions between

the viral and cellular sequences mediated by CTCF were proposed as a plausible mechanism contributing to the malignant transformation of CD4$^+$ T cells, while maintaining an architecture favorable for the virus.

CHROMATIN AND TRANSCRIPTION—INSIGHTS FROM RETROVIRUSES

The plasticity of the cellular genome is reflected in its ability to change in response to external stimuli. Acute viral infections like influenza often cause robust changes in cellular transcription programs. On the other hand, latent viral infections, typical for herpesviruses or lentiviruses, do not involve drastic changes in cellular transcription programs. They persist for longer periods while retaining the capability to be reversed and require different levels of precise transcriptional and chromatin control.

The HIV-1 transcriptional program consists of both phases with multiple layers of control. The viral LTR regulatory region has a structure of a typical RNAPII promoter with adjacent regulatory elements for binding of cellular TFs like nuclear factor (NF)-κB, nuclear factor of activated T cells (NFAT), or AP-1. In the basal, steady state, short abortive transcripts are transcribed from the LTR resulting in the establishment of latency (Shukla et al. 2020). One peculiarity of HIV-1 transcriptional control is the virally encoded Tat *trans*-activator (for a comprehensive review on viral TFs, see Ho et al. 2020) that binds to a *cis*-acting RNA element (*trans*-activation-responsive regions [TARs]) at the 5′ end of viral mRNAs. This promotes the assembly of a transcriptionally active complex containing RNAPII and positive transcription elongation factor complex (P-TEFb) (Zhu et al. 1997). Tat protein has been crucial for the discovery of the cyclin T1 component of P-TEFb (Wei et al. 1998), as for many other RNAPII elongation regulators and factors, like Spt5, DRB sensitivity-inducing factor (DSIF)/negative elongation factor (NELF), or BRD4 (for review, see Karn and Stoltzfus 2012).

Chromatin organization of proviral HIV-1 is rapidly established at the viral promoter upon integration and comprises three precisely positioned nucleosomes (nuc-0, nuc-1, and nuc-2)

with two intervening DNase I hypersensitive sites (DHSs) (Verdin et al. 1993). Among chromatin factors recruited to viral promoter, BRD4 stands out, as it encodes two isoforms with different roles in HIV-1 transcription. A long isoform contains a proline-rich terminal tail that binds P-TEFb (Wu and Chiang 2007) and supports viral transcriptional elongation (Jang et al. 2005; Yang et al. 2005; Bisgrove et al. 2007), whereas the short BRD4 isoform acts together with chromatin remodeling BAF complexes to silence HIV-1 (Conrad et al. 2017).

HIV-1 LTR nucleosome remodeling fine tune replication of the integrated viruses (Verdin et al. 1993; Lusic et al. 2003; Zhang et al. 2007; Natarajan et al. 2013). However, unintegrated extrachromosomal forms of HIV-1 are also chromatinized (Geis and Goff 2019), but unlike the integrated one they are loaded with repressive histone modifications, H3K9me3, and deprived from active marks (i.e., histone acetylation) (Geis and Goff 2019). MNase-seq and in silico predictions suggest that chromatinization into an additional nucleosome that covers DHS (nuc-DHS) occurs even before the integration process and is lost upon viral insertion (Machida et al. 2020). The existence of nuc-DHS is proposed to serve to silence viral DNA prior to becoming an integral part of the host genome.

Similar to the HIV-1, the MLV genome is also rapidly loaded with histones in the unintegrated form (Wang et al. 2016). The episomal MLV is often transcriptionally silent but can be reactivated upon treatment with histone deacetylase inhibitors, strengthening the notion that these forms are subjected to particular modes of epigenetic silencing (Wang et al. 2016). A CRISPR-Cas9 screen on cells infected with wild-type and integration-defective MLV showed the involvement of a histone-modifying complex in this silencing. The complex contains a DNA-binding protein with a zinc finger motif NP220, SET domain bifurcated histone lysine methyltransferase 1 (SETDB1), and the members of the human silencing hub (HUSH) complex (Zhu et al. 2018).

Primarily, the HUSH complex was shown to mediate position effect variegation (i.e., silencing

of a reporter construct as a result of its integration into silent chromatin) (Tchasovnikarova et al. 2015). It contains three main components: Family protein with sequence similarity (FAM208A), later renamed to transgene activator suppressor (TASOR), matrix metalloproteinase MPP8, and periphilin 1 (PPHLN1). It interacts with histone methyltransferase SETDB1 and is recruited to genomic loci enriched in H3K9me3 (Tchasovnikarova et al. 2015; Timms et al. 2016). Although HUSH activity was demonstrated on both MLV and HIV-1 promoters, individual knockout of the complex members does not affect HIV-1 expression. HUSH inefficacy in repressing HIV-1 might be in the viral genome code, as two studies point to the antagonist effects of viral protein Vpx in HUSH silencing (Geis and Goff 2019).

Among other important chromatin-binding factors, discovered or predominantly studied in the context of lentiviruses, is LEDGF/p75, a ubiquitously expressed chromatin reader and binding partner of HIV-1 IN (Cherepanov et al. 2003; Demeulemeester et al. 2015). LEDGF interacts with functionally diverse cellular factors and is involved in a broad range of nuclear mechanisms, at the crossroads of DNA repair, transcriptional control, and splicing (Aymard et al. 2014; LeRoy et al. 2019; Ui et al. 2020). Thanks to its carboxy-terminal IN-binding domain (IBD) and a chromatin-binding PWWP domain, LEDGF/p75 tethers HIV-1 PIC to chromatin (Demeulemeester et al. 2015). The PWWP domain binds specifically H3K36 trimethylated chromatin, enriched in the transcriptionally active regions of chromatin (van Nuland et al. 2013). As multiple splicing factors found to be LEDGF/p75 interacting partners (Singh et al. 2015) also bind to those regions, splicing determines LEDGF/p75 distribution on the genome and HIV-1 integration patterns (Francis et al. 2020). The promiscuous nature of LEDGF/p75 adds further to HIV-1 control by being involved in transcriptional repression and reactivation. Because of the interactions with the Spt6/ISWI complex, where depletion of all three components reactivates dormant HIV-1, LEDGF/p75 was proposed to play a role in HIV-1 latency (Gérard et al. 2015). By interacting with Pol II–associated factor (PAF1) and mixed lineage leukemia (MLL1), LEDGF/p75 can respec-

tively promote or reactivate the latent state (Gao et al. 2020).

Retroviral (M-MLV) proviral silencing in embryonic stem cells has been attributed to the concerted action of tripartite motif family (TRIM) 28 (Wolf and Goff 2007) and zinc finger protein (ZFP) 809 (Wolf and Goff 2009), which in turn recruit heterochromatin protein 1 (HP1) and H3K9 methyltransferases to sustain silencing. TRIM28, as well as TRIM33, were shown to have a role in the HIV-1 life cycle, regulating both integration and latency. TRIM28 binds the acetylated form of HIV-1 IN, mediates its deacetylation, and thus decreases the affinity of IN for DNA, resulting in impaired integration (Allouch et al. 2011). Its role in silencing of HIV-1 was attributed to its E3 small ubiquitin-like modifier (SUMO) ligation activity, which mediates CDK9 SUMOylation and interferes with its binding to cyclin T1 (Ma et al. 2019). E3 RING ligase activity of TRIM33 defines the stability of HIV-1 IN, therefore influencing the levels of integrated and produced viral progeny (Ali et al. 2019).

NUCLEAR BODIES

Given the densely populated nature of the nucleus, its intracellular organization is quite complex and comprises a number of nuclear bodies (NBs) with distinct functions such as promyelocytic (PML) NBs, splicing speckles, Cajal bodies, and nucleoli (Mao et al. 2011). Here we present an overview of spatial interactions and functions of the two most-studied NBs in the context of infections, PML NBs, and splicing speckles (Fig. 3).

PML NUCLEAR BODIES (PML NBs)

PML protein or TRIM19 is one of the most studied members of the TRIM family, ubiquitously expressed cellular factors with important roles in antiviral defense. Multiple cellular functions of PML are partially attributed to the fact that they interact with a variety of cellular factors (Van Damme et al. 2010) forming PML NBs. PML NBs have multifaceted roles in viral replication and persistence (Everett and Chelbi-Alix
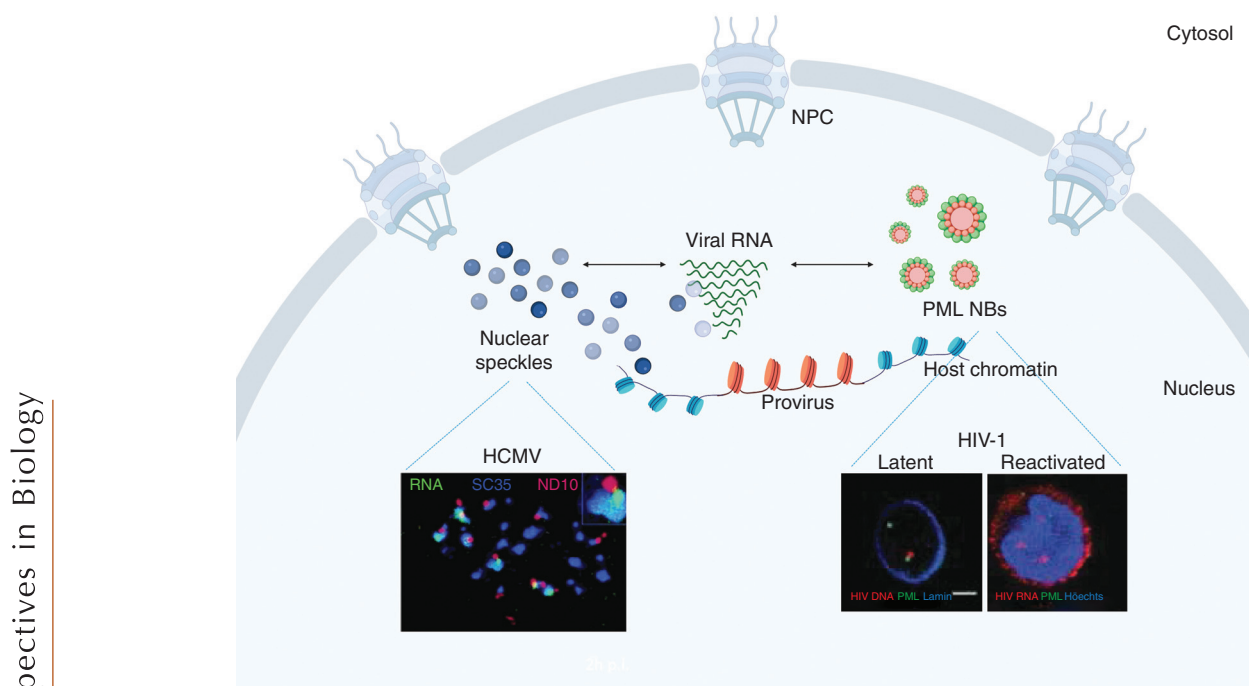
Figure 3. Interaction of viral genetic material with nuclear bodies (NBs). (*Left* panel) Human cytomegalovirus (HCMV)-infected cell triple-labeled with ND10 (promyelocytic [PML]) in red, IE transcripts in green, and SC35 in blue. Transcript signals appear to locate with the highest concentration at ND10 and are also found in the SC35 domain. (*Inset*) Arrangement as expected when all three components colocalize. (*Inset* preprinted from Ishov et al. 1997 with permission from Rockefeller University Press © 1997.) (*Right* panel) Images show HIV-1 DNA fluorescence in situ hybridization (FISH) (red) associated with promyelocytic (PML) nuclear bodies (NBs) (scale bar, 2 μm), (green) in latent cells and HIV-1 transcripts, RNA FISH (red) in activated cells where no PML NBs are detected. (*Right* panel from Shytaj et al. 2020; reprinted, with permission, from the authors in conjunction with the terms of the Creative Commons BY 4.0 license.)

2007; Scherer and Stamminger 2016). They arise via different pathways ranging from self-organization to ordered assembly with PML, a cysteine-rich, redox-sensitive protein, being their main nucleating component (Kamitani et al. 1998). PML is modified by SUMOylation in response to reactive oxygen species, which together with recruitment of its partner proteins via the SUMO-interacting motif (SIM), mediate the nucleation of NBs (Sahin et al. 2014). The putative antiviral properties of PML have been attributed to its IFN pathway-regulatory role, as it is one of the main targets of IFN signaling (Lavau et al. 1995). Whereas many viruses act to induce the reorganization and/or degradation of PML NBs, many DNA viruses establish viral replication factories (VRCs) in their proximity.

This highlights the dual nature of PML NBs as both repressors and supporters of proviral functions (especially viral latency) (Charman and Weitzman 2020).

PML NB-mediated intrinsic defense represents an important event in replication of all herpesviruses, influencing both the lytic and latent phase of their life cycle. In HSV-1 infection, infected cell protein 0 (ICP0), a viral ubiquitin ligase with multiple SIM-like sequences, promotes viral gene expression and reactivation from latency (for review, see Rodríguez et al. 2020). ICP0 uses both SUMO-dependent and -independent targeting strategies to ubiquitinate and degrade PML and Sp100, events that result in disassembly of PML NBs and release of viral genomes to ensure viral replication (Lee et al.

2004). Furthermore, almost immediately upon nuclear entry, HSV-1 genomes colocalize with another component of PML NBs, the SWI/SNF chromatin remodeler protein, α-thalassemia X-linked intellectual disability (ATRX), that together with histone chaperone and PML NB-resident death domain–associated protein 6 (Daxx) restricts viral expression by stabilizing the maintenance of viral heterochromatin (Cabral et al. 2018).

Human cytomegalovirus (HCMV) localizes adjacent to PML NBs both before establishing VRCs and after, as PML NBs support spatiotemporal immediate transcription (Ishov and Maul 1996; Ishov et al. 1997). Viral immediate early 1 (IE1) protein interacts with PML protein and colocalizes in PML NBs preventing its oligomerization, reducing de novo PML SUMOylation and ultimately leading to their disruption (Tavalai and Stamminger 2011).

The IE1 protein human herpesvirus 6B (HHV-6B), also encoded by the closely related betaherpesvirus, colocalizes with PML and SUMO-1 during infection without inducing their dispersion (Gravel et al. 2002). SUMOylation of IE1 occurs at telomeres where PML NBs are located (Collin et al. 2020), and this was proposed to represent an advantage for maintenance of viral latent state (Arbuckle et al. 2010).

In latent infections with EBV that were connected with nasopharyngeal carcinoma, EBNA1 viral protein disrupts PML NBs, thus impairing DNA damage response and compromising cell survival (Sivachandran et al. 2008). Insights from the herpesviruses biology clearly underline the multifaceted role of PML in infection and suggest that the closely related viruses of this family could both exploit and oppose PML nuclear compartments to ensure viral progeny. At the same time, herpesviruses show how viruses coevolved with their hosts to control cellular repression mediated by components of PML NBs (Everett et al. 2013).

Adenovirus infections display perhaps the most compelling illustration for the PML NB-related rearrangements upon viral nuclear entry. Two early expressed viral proteins, E4-ORF3 11 kD and E1B 55 kD, are targeted to PML foci that are in turn reorganized into striking nuclear track-like structures, thus disrupting PML NBs (Doucas et al. 1996; Berscheminski et al. 2014). E4-ORF3 assembly creates avidity-driven interactions with PML, offering a possible mechanism for the disruption of PML NBs and other tumor suppressor complexes (Ou et al. 2012). PML NB components Daxx and ATRX mediate an epigenetic repression of the immediate early adenoviral promoter (Schreiner et al. 2013), while viral CA protein VI efficiently counteracts this host cell defense system (Schreiner et al. 2012). Other NB protein residents, such as Sp100 and NDP55, are redistributed at the sites of VRCs (Doucas et al. 1996; Berscheminski et al. 2014), suggesting that adenovirus could both suppress the cellular defense mechanisms by sequestering the cellular factors, or these factors may be exploited for viral replication.

PVs use this cellular SUMOylation system at distinct stages of their life cycle and associate with PML NBs. Early studies on bovine papillomavirus (BPV) suggested that PML NBs are necessary for the establishment rather than for the restriction of infection (Day et al. 1998). HPV CA protein was then implicated in the reorganization of PML NBs in natural productive lesions (Florin et al. 2002). During mitotic membrane breakdown, viral genomes localize to PML NBs already during viral early gene transcription (Day et al. 2004). Homing to PML NBs is mediated by SUMOylation of L2 CA, an event indispensable for efficient infection (Marušič et al. 2010; Bund et al. 2014). Of note, HPV L2 presence up-regulated the overall SUMOylation status of the host cell, similarly to what is observed upon HIV-1 infection in $CD4^+$ T cells (Lusic laboratory, unpubl.). A recent high-resolution imaging study showed that rather than the virus targeting NBs, PML, SUMO-1, and Sp100 proteins are recruited and assembled around the papilloma viral genomes early in the interphase (Guion et al. 2019). Hence, PML NBs could provide a protective habitat until switching from productive to latent infection.

HIV-1 establishes multivalent interactions with PML NBs, which affects HIV-1 replication and persistence (Marcello et al. 2003; Lusic et al. 2013; Shytaj et al. 2020). Early studies suggested that PML protein sequesters cyclin T1 (part of

P-TEFb elongation complex) into NBs, abrogating HIV-1 transcription (Marcello et al. 2003). Subsequently, PML NBs were shown to associate with integrated but transcriptionally silent viral genomes (Fig. 3, right insets). PML transcriptional repression was shown to be exerted through direct interaction with G9a histone methyltransferase and H3K9me2 deposition, a facultative heterochromatin mark within euchromatic regions (Lusic et al. 2013). Reports of atypical PML NBs translocation to the cytoplasm during early viral events and association of Daxx protein with incoming virions followed by inhibition of HIV-1 reverse-transcription point to the multiple roles of PML NBs in restricting retroviruses (Dutrieux et al. 2015).

Multiple layers of complexity are reflected in the fact that metabolic changes in T cells caused by retroviral replication and reactivation from latency are sensed at the level of PML NBs. HIV-1 replication results in up-regulation of oxidative stress response and by nuclear factor erythroid 2–related factor 2 (NRF2) translocation to the nucleus. The activation of antioxidant pathways results in reversible reduction in PML NBs via SUMO-mediated proteasomal turnover. With reactivation of cells, viral RNA becomes detectable in the nuclei, but very little or no PML bodies can be seen (Fig. 3, right insets). The reformation of PML NBs, when viral transcription ceases, contributes to the establishment of HIV-1 latency (Shytaj et al. 2020). These findings place PML NBs in the spotlight of cellular immune-metabolic changes caused by the pathogen, and open a possibility that PML NBs could act as metabolic stress sensors and serve as markers and/or pharmacologic targets of HIV-1 infection.

NUCLEAR (SPLICING) SPECKLES (NS)

Nuclear (or splicing) speckles (NS) are known to be enriched in factors related to transcriptional pause release, RNA capping and splicing, polyadenylation and cleavage factors, and RNA export (Galganski et al. 2017). The current model of NS organization postulates that SC35 (serine/arginine-rich splicing factor [SRSF]2) and SON proteins localize in the central regions with

RNAs enriched at the periphery. SON, also known as negative regulatory element-binding protein, has initially been described as a factor-binding DNA sequence upstream of the core promoter and second enhancer of HBV, and was shown to repress HBV core promoter activity (Sun et al. 2001). Its role as a speckle scaffolding protein (Sharma et al. 2010) was highlighted when SON was used in TSA-seq (tyramide signal amplification) to define speckle-associated domains (SPADs) (Chen et al. 2018). Based on TSA-seq and other two recent genome-wide technologies, 50% of all protein-coding genes are now considered to colocalize with NS (for review, see Chen and Belmont 2019). The functional significance of this localization was probed using the HSP70 gene (HSPA1A) as a model, shown to move toward the speckles upon transcriptional activation. Intriguingly more than two decades ago, HCMV was used to ask similar questions, namely, the location of induced genes (Fig. 3, left inset; Ishov et al. 1997). In case of HCMV, transcription starts in proximity of PML NBs, from where transcripts move toward the spliceosome assembly domain (SC35 domain). Immediate early viral proteins IE72 and IE 86, which synergistically activate viral and cellular gene expression, are the ones that modulate NBs. IE72 interacts with PML NBs, while IE86 accumulates at PML NBs, which are in the proximity of SC35 domains.

Although NS are considered sites of storage/modification of splicing factors, until recently it was not entirely clear whether splicing indeed occurs at these sites. Recent evidence obtained on examples of cellular genes (Dias et al. 2010; Girard et al. 2012) are strongly supported by data obtained on influenza virus. Evidence for association with NS, splicing, and subsequent export are provided on the example of IAV mRNA, where viral intronless M1 mRNA is targeted to NS by viral protein NS1. SON protein and members of the export TREX complex are then involved in the splicing and subsequent export of viral mRNA to the cytosol (Mor et al. 2016).

HIV-1 genome generates more than 50 mRNA variants from its nine genes, and despite these splicing events, interactions with speckles have so far been attributed mostly to the viral Tat

protein and to its interaction with P-TEFb (Herrmann and Mancini 2001; Marcello et al. 2001). Interestingly, very recent findings point to the fact that a portion of HIV-1 insertions into the cellular chromatin occurs in SPADs. This targeting is dependent on the host factor CPSF6, which binds HIV-1 CA during PIC income, possibly targeting the virus to transcriptionally active regions that are in close proximity to NS sites (Francis et al. 2020). Moreover, a large part of genomic regions mapped closest to NS fall into the A1 chromatin compartments, which are enriched in SE, consistent with the recent findings on HIV-1 integration into these portions of the genome (Chen et al. 2017; Lucic et al. 2019).

CONCLUDING REMARKS

To be able to exert a plethora of functions, the cellular genome self-organizes in a hierarchical manner. Nonrandom but highly variable patterns that govern the organization of the mammalian nucleus, although seemingly opposite, ensure all nuclear functions. These patterns of organization are adopted by viruses that complete their life cycle in the nucleus. At the same time, viruses affect architectural robustness and pose challenges to the plasticity of the genome. They have evolved multiple strategies to enter the nucleus, to explore cellular chromatin by episomal contacts or by direct genomic integrations, and to either disrupt or use NBs as shields for their own replication and persistence.

As one of their main features is to explore and use cellular mechanisms, they have been very lucrative tools for studying different nuclear functions, thus paving the way for many important discoveries. The fact that new viruses with critical implications for human health are still emerging highlights the importance of viral research that will continue to move boundaries in our understanding of many previously unknown nuclear and more broadly cellular organization features.

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