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Jumping over the fence

RNA nuclear export revisited

Caterina Strambio-De-Castilla

Program in Molecular Medicine; University of Massachusetts Medical School; Worcester, MA USA

The nuclear envelope forms a cocoon that surrounds the cellular genome keeping it out of harm's way and can be utilized by the cell as a means of functionally regulating chromatin structure and gene expression. At the same time, this double-layered membrane system constitutes a formidable obstacle to the unimpeded flow of genetic information between the genome and the rest of the cell. The nuclear pore has been long considered the sole passageway between nucleus and cytoplasm. A new report[1] challenges this view and proposes a novel mechanism by which RNA transcripts destined for localized translation in highly polarized cell types, cross both inner and outer nuclear envelope membranes and reach the cytoplasm without utilizing the nuclear pore route.

Keywords: nuclear envelope, nuclear lamina, nuclear pore complex, Wnt signaling, neuromuscular junctions, RNA export

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Correspondence to: Caterina Strambio-De-Castilla; Email: caterina.strambio@umassmed.edu

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Compartmentalization Creates Barriers

Although compartmentalization affords eukaryotic cells complex means of functional regulation, it also poses considerable logistical challenges arising by the need to exchange material between separate organelles. The two major cellular compartments are separated by the nuclear envelope (NE), a double membrane system composed of an inner nuclear membrane (INM) facing the nucleoplasm and an outer nuclear envelope (ONM), which faces the cytoplasm and is continuous with the endoplasmic reticulum.[2] While the NE and its associated structures constitute a formidable barrier, protecting the cellular genome against external threats, its presence also poses a significant obstacle to the physiological exchange of information between the genome and the rest of the cell.

In multicellular organisms, the interface between chromatin and the INM is occupied by the nuclear lamina (NL), a 30–100 nm thick, dense protein meshwork, which has an essential role in preserving both the shape and the mechanical properties of the nucleus.[3,4] The NL is composed of four lamin proteins, which are subdivided in types A and B and collectively belong to the type V intermediate filaments family. The INM-associated type B lamins (i.e., lamin B1 and B2) are the fundamental lamina building blocks, while the nucleoplasm-facing type A lamins (i.e., lamin A and C), have more specialized functions. Besides its scaffolding and protective roles, it is now increasingly clear that the NL represents a hub for the coordinated interaction between macromolecular machineries involved in multiple cellular functions. These include gene regulation, genome organization and repair, as well as mitotic division, nuclear positioning, cytoskeletal remodeling and nucleocytoplasmic transport.[5,6] Not surprisingly, given its far ranging and pivotal roles, lamina defects have been associated with a variety of human disorders, collectively termed laminopathies, which include muscular dystrophy, cardiomyopathy and progeroid syndrome.[7,8]

The Canonical View of Nuclear RNA Export Has Its Difficulties

Given such an apparently inexpugnable fortification, it is not surprising that

nature has devised specialized pathways to ensure efficient material exchange in and out of the nucleus. Most traffic across the NE and lamina barriers is accomplished through cylindrical macromolecular assemblies termed nuclear pore complexes (NPCs). Among the largest proteinaceous machineries in the cell, these structures are highly selective molecular sieves, constructed of multiple copies of ~30 different component proteins termed nucleoporins, or Nups. NPCs lie in apertures that are evenly distributed on the plane of the NE, traverse both INM and ONM and are tightly embedded within the nuclear lamina through interactions between type A lamins and specific Nups.[3,9] While their main function is that of "toll booths" regulating the traffic of both small molecules and large macromolecules in an out of the nucleus, a growing body of evidence suggests their involvement in many other cellular activities, including the epigenetic regulation of gene expression and chromatin maintenance.[10,11] Finally, NPCs also likely contribute to the cohesion between INM and ONM, as testified by the major NE structural alterations observed in some Nup mutant yeast strains.[12,13]

The metazoan NPC consists of a ~125 nm diameter core structure composed of three main rings surrounding a central transport channel. The diameter of this channel has been inferred from the size of un-deformable artificial cargo[14] as well as from direct measurements[15] and it ranges between ~40 nm near its midplane to ~60–70 nm at either end. While much has been learned on the structure and function of the NPCs since their initial observation,[16,17] one outstanding question has concerned the mechanism by which large ribonucleaoprotein (RNP) assemblies gain access to the cytoplasm, when their diameter can be considerably larger than the diameter NPC central transporter.[18] The generally accepted model has been largely based upon observations of *Chironomus* Balbiani ring mRNP granules (diameter ~50 nm) and of ribosomal large subunits (diameter ~30 nm) export and posits that large RNPs are temporarily rearranged into more elongated structures during nuclear egress and are threaded piecemeal through the NPC central channel.[19-24]

While this current unfurling model is tempting, it presents several potential challenges.[25] One obvious concern pertains the size limit for the efficient export of multimegadalton RNP assemblies from the nucleus. A second, related question regards whether this model could account for the transport efficiency required during active gene expression. Finally, a particularly intriguing challenge concerns the nuclear export of transcripts known to undergo delayed localized translation at specialized cellular sites.[26,27] Unfolding Balbiani ring granules have been observed to engage ribosomes while still in transit through the pore.[28] This rapid engagement by the cytoplasmic protein synthesis machinery would clearly interfere with the ability of specialized mRNAs to delay translation until after they have reached their designated cellular locale.

The Escape Pathway Utilized by Herpes Viruses Bypasses the NPC

One way to investigate the mechanisms governing the transport of large nucleoprotein complexes in and out of the nucleus is to study the path followed by those viruses that utilize the nucleus as their site of replication. While many viruses avoid the nucleus altogether, several families of DNA viruses as well as the lentiviruses, need to cross the NE both on their way in and out of the nucleus.[29] In most described cases this occurs via the NPC. One notable exception is represented by the pathway utilized by the herpes simplex virus (HSV) nucleocapsid to exit the nucleus. According to the now widely accepted NE budding model,[30] the newly assembled, 115–130 nm diameter wide viral capsid first moves from the sites of assembly toward the nuclear periphery and makes contact with the NL. Subsequently, the recruitment of cellular kinases mediates lamin phosphorylation, which in turn is thought to lead to local disruptions of the lamina network. Once the lamina barrier is dissolved, the nucleocapsid can enter in direct contact with the INM and bud into the perinuclear space. Following this primary envelopment event, a "naked" HSV capsid is released to the cytoplasm through a fusion event between its primary envelope and the ONM. Following de-envelopment, the nucleocapsid follows a complex series of steps that eventually lead to viral release.

Expectedly, in order to facilitate this complex nuclear "evasion" mechanism the NE structure faces major perturbations during the course of HSV infection. Consistent with A- and B-type lamins site-specific phosphorylation by protein kinase C (PKC), lamina components undergo major conformational and localization alterations leading to localized lamina dissolution.[30] In addition to the rearrangement of the lamina, several documented instances of NPC clustering and gross enlargements have also been reported.[31-33] Strikingly however, even in cells where nuclear pores were observed to be ten times as wide as their normal diameter, HSV capsids were never "caught" while "escaping" through the NPC, suggesting that more work has to be done to understand the role of canonical nucleocytoplasmic transport in HSV egress.[34] One obvious possibility is that the observed effects on NPC size and spacing are secondary to the disruption of the lamina network and might be a pre-requisite for NE budding. For example, it is easy to imagine how increasing the inter-NPC distance might facilitate the budding process by expanding the area of available NPC-free regions of the INM and by reducing the capacity of NPCs to staple the inner and the outer NE layers together.

Nuclear Export of Large RNPs Destined for Localized Translation Follows the HSV Route

Viruses have often proved invaluable tools for unearthing previously undetected cellular processes. Recent studies into the mechanisms governing the nuclear export of large mRNP assemblies destined for localized protein synthesis during Drosophila larval development[35] might represent once again a case in point. In this system, the rapid morphogenesis of synaptic boutons that occurs in response to motor neuron signaling during neuromuscular junction (NMJ) formation, appears to occur via the coordinated transport of large quantities of bouton-specific transcripts to the patterning site

followed by their localized translation.[35] Building on this scenario, a recent report by Speese et al.[1] challenges the canonical view of RNA export and presents compelling evidence for an alternative pathway that bypasses the NPC altogether.

Studying Wnt signaling in post-synaptic muscle fibers, Budnik and colleagues had previously revealed that a C-terminal fragment of the Dfrizzled2 Wnt-1 receptor (DFz2C) is imported into the nucleus in response to synaptic stimulation, forming discrete peripheral nuclear foci.[36,37] In this new study, the authors show that once inside the nucleus, DFz2C localizes in large (i.e., ~200 nm in diameter) electron-dense granules containing bouton-specific mature. RNA transcripts poised for nuclear export. Such granules accumulate on the nuclear face of the NE where they appear to be encased within scaffolds composed of the A-type lamin, laminC (LamC) and to be surrounded by membranes. Interestingly in addition to DFz2C, both lamC and atypical protein kinase C (aPKC) are required for the formation of these granules, and in their absence NMJ development is hampered. The involvement of aPKC suggests that the morphogenesis of the DFz2C/LamC granules requires the reorganization of the lamina and NE structures, as observed during HSV NE budding. Consistent with this conclusion, high-resolution microscopic images indicate that the DFz2C/LamC-granules are enveloped by NE invaginations that appear to be continuous with either the INM or the ONM and keep the granular content topologically separate from the cytoplasm. Taking these observations and the sheer size of the DFz2C-granules together, the authors propose a model by which their nuclear export occurs via a NE budding process akin to the one employed by the capsid of HSV to exit the nucleus.[30]

NE Budding Might Be the Answer

While the model proposed by Speese et al. is clearly compelling, much remains to be resolved. For example, one open issue regards both the rationale and the universality for such a potentially disruptive mechanism for the export of large endogenous aggregates from the nucleus.[38] The need to efficiently and synchronously export large quantities of transcripts destined for localized translation, clearly warrants the idea of a dedicated transport mechanism that preserves cohesion among multiple mRNA moieties during coordinated cytoplasmic trafficking, while at the same time preventing premature exposure to the protein synthetic apparatus. Nevertheless, it is difficult to envision a universal mechanism of nuclear export that entails "punching holes" through the NE and lamina wall instead of utilizing readily available and abundant pre-made doorways that safely interrupt the NE and the lamina without affecting nuclear stability and function. While instances of NE "blebbing" associated with nucleocytolasmic exchange have been described,[39,40] most reports are confined to early embryogenesis or other developmental stages, raising the possibility that this alternative mechanism for the export of multimegadalton RNP assemblies might be confined to specific cell types requiring bursts of efficient and localized protein synthesis to support rapid differentiation and patterning events. Consistent with this view, unconventional NE structures associated with specific developmental stages have been previously reported.[41]

Most important, the cellular molecular machinery involved in NE budding remains to be dissected. For instance, while the authors have established a role for DFz2C, LamC and aPKC in the formation of DFz2C granules and in NMJ patterning, the molecular role of these components remains to be established. In addition, further studies are warranted to uncover other players in the NE budding pathway. Obvious candidates are INM components, such as Emerin, and lamin B receptor (LBR) and the Torsin AAA+ ATPase, which were shown to have roles in the exit of hersviruses from the nucleus.[30] More in general it will be important to determine what membrane deformation system is employed here to enable the complex series of membrane fission and fusion steps required for NE budding. In this context, it is interesting to notice that pore membrane hugging components of the NPC scaffold, are structurally related to components of the clathrin, COPI and COPII vescicle-coating complexes.[42] Is it possible that these Nups might have a role here in promoting membrane bending during NE budding? Furthermore, could the NPC and the NE budding nuclear export pathways in fact be evolutionarily related?[43] Data presented in Speese et al. is consistent with the idea that sites where the DFz2C granules initially associate with the NE are in spatial proximity to NPCs. This observation should not be dismissed. Rather it should be followed up with dynamic imaging, genetics and ex vivo interaction studies to assess the involvement of individual NPC components in the described NE budding mechanism.

A related question centers on the availability of sufficient NPC-free areas in the INM of larval Drosophila nuclei, to allow budding.[44,45] This is relevant because the presence of closely spaced NPCs in the plane of the NE is expected to hamper the capacity of the two NE membrane-layers to come apart and allow invagination and subsequent evagination events such as the ones required to permit the proposed budding pathway. During HSV infection, NPCs have been shown to change their distribution in the plane of the membrane, presumably facilitating NE blebbing. Thus, it will important to establish what is the average steady-state inter-NPC distance in the system under study here and whether this spacing is affected by Wnt signaling similar to what observed during HSV nucleocapsid nuclear egress.[30]

Finally, other open questions ask what is the ultrastructural organization and composition of the DFz2C/LamC granules. The authors convincingly show by different high-resolution imaging techniques that LamC and nuclear membranes encase electron-dense granules containing both DFz2C and bouton-specific mRNAs. Nonetheless, their internal structure and molecular composition remains to be established. Presumably they consist of aggregates of mRNPs encoding bouton components. It will be interesting to determine how many different transcript species can be shown to aggregate in the same granule, how multiple mRNPs might interact with each other to form such large electron-dense assemblies and whether their structure is amorphous or highly-ordered. Regardless of their internal structure, it will be important to extend the

imaging findings presented here by high-resolution time-lapse microscopy, by serial sectioning EM coupled with 3D image reconstruction,[46,47] as well as by correlative light-electron microscopy.[48] These experiments will allow to further investigate the morphogenesis of the multimegadalton complexes following Wnt signaling, their movement inside the nucleus and their spatial relationship with both the NE and the NPC. Similarly, it will be important to establish whether the NE bulges observed in this system bear any structural resemblance of functional relation to similar non-canonical NE structures observed in other systems.[12,13,49,50]

Despite these caveats, the prevailing fact remains that the pioneering work recently presented by Speese et al. has shed unexpected light into previously uncharted territory. Their effort will now hopefully be followed up by them and by others and lead to a better understanding of the means by which the two main compartments of the eukaryotic cell can effectively communicate with each other across the NE barrier, especially during active growth and development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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