Jumping over the fence: RNA nuclear export revisited

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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 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.

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. 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. 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. 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.

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 nucleoporins, as well as from direct measurements and temporally rearranged into more elongated structures during nuclear egress and postulated by reduced the capacity of NPCs to staple the inner and outer NE layers together.

The Escape Pathway Utilized by Herpes Viruses Bypasses the NPC

One way to investigate the mechanisms governing the transport of large nucleo-protein 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 documented instances of NPC clustering and gross enlargements have also been reported. 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. One obvious possibility is that the observed effects on NPC size and spacing are secondary to the disruption of the lamina network and not 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 outer NE layers together.

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

Viruses have often proved invaluable tools for unraveling 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 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.36 Building on this scenario, a lost 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 a Wnt-1 receptor (DFz2C) is imported into the nucleus in response to synaptic stimulation, forming discrete peripheral nuclear foci.30,31 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 continuous with either the INM or the ONM.

The need to efficiently and synchronously export large quantities of transcripts designed for localized translation, clearly warrants the idea of a dedicated transport mechanism that preserves cohesion among multiple mRNA species 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 nucleocytoplasmic 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.11

Most important, the cellular 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 Torin AAA+ ATPase, which were shown to have roles in the exit of retrovirus from the nucleus.12 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 vesicle-coating complexes.13 Is it possible that these Nups might have a role here in promoting the pore binding during NE budding? Furthermore, could the NPC and the NE budding nuclear export pathways in fact be evolutionarily related? 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. 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.13,36 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 viral egress.16 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 many 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

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 imaging findings presented here by high-resolution light-electron microscopy.46,47 These experiments will allow us 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 reconstruct, as well as by correlative sectioning EM coupled with 3D imaging. Findings presented here by high-resolution light–electron microscopy.48 These experiments will allow us 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 bears any structural resemblance of functional relation to similar non-canonical NE structures observed in other systems.49-51

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.

References


40. Gar H. Nucleocytoplasmic relations in Drosophila.


44. Wray M, Yarar D, Goldberg TH Jr, Martinez-Arias AS. Nuclear pore complex number and distribution throughout the Saccharomyces cerevisiae cell cycle by three-dimensional reconstruction from electron micrographs of nuclear envelopes. Mol Biol Cell 1997; 8:1419-14; PMID:9620877.


