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. 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, cytoskeletal remodeling and nucleocytoplasmic transport. 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.

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 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, controlling specific barriers observed in the NE structural alterations are tightly embedded within the nuclear lamina through interactions between type A lamins and specific Nups. While their primary function is that of "tollbooths" regulating the traffic of both small molecules and large macromolecules in and 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. 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. The metazoan NPC consists of a ~325 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 as well as from direct measurements 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, one outstanding question has concerned the mechanism by which large ribonucleoprotein (RNP) assemblies gain access to the cytoplasm, when their diameter can be considerably larger than the diameter NPC central transporter. 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 unraveled into more elongated structures during nuclear egress and are threaded piecemeal through the NPC central channel. While this current unfolding model is tempting, it presents several potential challenges. 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 putative model of viral nuclear export of transcripts known to undergo delayed localized translation at specialized cellular sites. Unfolding of the viral nucleocapsid is thought to lead to local ribosome assembly and translation of viral mRNAs. While this current unfurling model has been largely based upon observations of Chironomus Balbiani ring mRNP granules, ribosomal large subunits and viral nucleocapsids, recent advances in understanding the mechanism by which large virions cross the NPC barrier have provided new insights into the nature of this process. For example, HSV nucleocapsids are observed to be ten times as wide as their normal diameter during nuclear egress. 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 the role of canonical nucleocytoplasmic transport in HSV egress is less clear. 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 understanding previously undetected cellular processes. Recent studies into the mechanisms governing the nuclear export of large RNP 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.35 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 the Wnt-1 receptor (DFz2C) is imported into the nucleus in response to synaptic stimulation, forming discrete peripheral nuclear foci.24,25 In this new study, the authors show that once inside the nucleus, DFz2C localizes in large (~200 nm in diameter) electron-dense granules containing MSNA-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 lamins, laminC (LamC) and to be surrounded by membranes. Interestingly in addition to DFz2C, both lamC and a putative 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 laminae 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.36-38

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.39 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 moieties during concerted 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,40,41 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.42

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 herpersvirus from the nucleus.43

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 core membrane-hugging components of the NPC scaffold, are structurally related to components of the clathrin, COPI and COPII vesicle-coating complexes.44 Is it possible that these Nups might have a role in membrane shaping, or fusion during NE budding? Furthermore, could the NPC and the NE budding nuclear export pathways in fact be evolutionarily related?45 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. However, 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.45-47 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.48 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 protein-SNA-specific mRNAs. Nonetheless, their internal structure and molecular composition remains to be established. Presumably they consist of aggregates of mRNA encoding coatomer components. It will be interesting to determine how many different transcript species can be shown to aggregate in the same granule, how multiple mRNAs 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
establish whether the NE bulges observed in this system bear any structural resemblance to similar non-canonical NE structures observed in other systems. 

Despite these caveats, the prevailing fact remains that the pioneering work recently presented by Spreer et al. has shed unexpected light into previously uncharted terrain. 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.

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