Jumping over the fence: RNA nuclear export revisited

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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.8 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.9,10 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.11-13 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.14,15

The Canonical View of Nuclear RNA Export Has Its Difficulties

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

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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, controlling the transport of 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 lamins and specific Nups. While their main function is that of “tollbooths” 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. Finally, NPCs also likely contribute to the cohesion between INM and ONM, as testified by the major lamins and specific Nups. Initially, an outstanding observation, one outstanding question has concerned the mechanism by which large ribonucleoprotein (RNP) assemblies gain access to the cytoplasm, when their diameter can be considered larger than the diameter NPC central transporter. The generally accepted model has been largely based upon observations of ChromosomaBalbiani ring mRNPs, and of ribosomal large subunits (diameter ~30 nm) and export mRNPs that large RNPs are temporarily rearranged into more elongated structures during nuclear egress and are threaded piecemeal through the NPC central channel.

While this current unfurling model governing the nuclear export of transcripts known 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 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 to exit the nucleus. According to the now widely accepted NE budding model, the newly assembled, 115–130 nm diameter 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 viral envelope. In eukaryotes, 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 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 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 to exit the nucleus. According to the now widely accepted NE budding model, the newly assembled, 115–130 nm diameter 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 viral envelope. In eukaryotes, the nucleocapsid follows a complex series of steps that eventually lead to viral release.

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followed by their localized translation. The need to efficiently and synchronously export large quantities of transcripts designated for localized translation, clearly warrants the idea of a dedicated transport mechanism that preserves cohesion among multiple mRNA moieties during continuous cytoplasmic trafficking, while at the same time preventing premature exposure to the protein synthetic apparatus. This observation is consistent with 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, 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.

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 lamina B receptor (LBR) and the Torin AAA+-ATPase, which were shown to have roles in the exit of herpesviruses from the nucleus.

More in general it will be important 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 issue regards both the rationale and the universality for such a potentially disruptive mechanism for the export of large endogenous aggregates from the nucleus. A related question centers on the availability of sufficient NPC-free areas in the INM of larval Drosophila nuclei, to allow budding. This is consistent with 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.

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 boun-ton-specific mRNAs. Nonetheless, their internal structure and molecular composition remains to be established. Presumably they consist of aggregates of mRNA encoding boun-ton 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

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.
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.23,24

Despite these caveats, the prevailing fact remains that the pioneering work recently presented by Speere et al. has shed new light into previously uncharted territories. Their efforts 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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