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Current Opinion in Cell Biology

This bud's for you: mechanisms of cellular nucleocytoplasmic trafficking via nuclear envelope budding

Lee G Fradkin and Vivian Budnik

The nuclear envelope (NE) physically separates the cytoplasmic and nuclear compartments. While this barrier provides advantages, it also presents a challenge for the nuclear export of large ribonucleoprotein (RNP) complexes. Decades-old dogma holds that all such border-crossing is via the nuclear pore complex (NPC). However, the diameter of the NPC central channel limits the passage of large cargos. Here, we review evidence that such large RNPs employ an endogenous NE-budding pathway, previously thought to be exclusive to the nuclear egress of Herpes viruses. We discuss this and other models proposed, the likelihood that this pathway is conserved, and the consequences of disrupting NE-budding for synapse development, localized translation of synaptic mRNAs, and laminopathies inducing accelerated aging.

Address
Department of Neurobiology, University of Massachusetts Medical School, 364 Plantation St, Worcester, MA 01605, USA

Corresponding author: Budnik, Vivian (Vivian.budnik@umassmed.edu)

Current Opinion in Cell Biology 2016, **41**:125–131

This review comes from a themed issue on **Cell Organelles**

Edited by **Pedro Carvalho** and **Davis Ng**

For a complete overview see the Issue and the Editorial

Available online 26th May 2016

http://dx.doi.org/10.1016/j.ceb.2016.05.001

Introduction

Envelopment of genomic material by a double membrane, a hallmark of eukaryotic cells, was first described over a hundred years ago [1] and likely first occurred some 3 billion years ago. The segregation of the nucleoplasm from the cytosol enabled the spatial-temporal separation of translation from transcription [2]. This, in turn, allowed the evolution of additional regulatory levels necessary for the development of complex multi-cellular organisms, e.g., RNA export and cytoplasmic trafficking, or spatially restricted post-translational control, such as phosphorylation, ubiquitinylation and sumoylation. The nuclear envelope (NE) is composed of an outer nuclear membrane (ONM), which is continuous with the endoplasmic reticulum (ER), and an inner nuclear membrane (INM) that faces the nucleoplasm. Beneath the INM there is a dense meshwork, the lamina, primarily comprising intermediate filament proteins, the Lamins. Far from representing a passive component of the nucleoskeleton, the Lamins play dynamic roles in nuclear mechanical stability, chromatin organization, regulation of gene expression, genome stability, and cell division and differentiation [3]. Lamins often exist in two flavors, A-type Lamins (encoded by the *LMNA* gene in mammals, which generates two isoforms, LMNA and LMNC, and the *lamC* gene in *Drosophila*), and B-type Lamins (encoded by *LMNB1* and *LMNB2* in mammals and *lamDm0* in flies). While B-type Lamins are expressed from the earliest stages of cellular differentiation and are required for cell viability, A-type Lamins are usually present in differentiated cells and are not required for cell survival. Indeed, animals such as *C. elegans* lack A-type Lamin, expressing only B-type Lamin. In humans a very large number of mutations (300+) in the *LMNA* gene have been identified, causing tissue-specific dystrophies and early aging, and collectively known as laminopathies [4].

Until recently it had been assumed that, except during early phases of mitosis, when the NE and lamina break down to be subsequently reassembled during telophase, the Nuclear Pore Complex (NPC) was the only gateway in and out of the nucleus. The NPC, first described over 50 years ago [5], is a ~125 MDa protein complex, with ~3000 of them spanning each higher eukaryotic cell NE. It consists of some 500 proteins, primarily multiple copies of ~30 distinct nucleoporins that form a central channel through which cargo is transported in and out of the nucleus in a highly-regulated fashion [6,7]. Both passive diffusion and facilitated transport, enabled by nuclear localization and export sequences within proteins and RNAs, as well as NPC-associated elements, are supported by the NPC.

A major problem with postulating the NPC as the sole route of nucleo-cytoplasmic transport is the relatively small size of molecular complexes that can transverse through its central channel. Studies using gold particles complexed to nuclear-localizing cargo of various sizes, used as molecular calipers to measure the size of the NPC central channel, placed its diameter at ~39 nm [8]. Moreover, recent structural studies indicate that the NPC channel is relatively flexible, being able to stretch up to 52 nm in diameter [9,10]. There has been, however, a significant number of studies in diverse eukaryotic cells

and tissues indicating the existence of much larger (100–700 nm) RNPs in the cytoplasm [11,12,13,14,15]. Given the NPC size constrains it has been proposed that these super-large RNPs are assembled in the cytoplasm and that RNAs exit the nucleus one by one through the NPC. A potential mechanism to explain the nuclear export of RNPs larger than the maximum diameter of the NPC central channel has been suggested in the study of Balbiani ring RNPs which are synthesized in the nucleus of larval salivary gland cells of the dipteran *Chironomus tentans*, with a ~50 nm in diameter. Within the nucleus, these RNPs form a ribbon which assumes a ring-like conformation [16]. During RNP extrusion through the NPC, they adopt an elongated conformation, enabling their passage through the NPC [16]. The transit of other 'oversized' RNPs from the nucleus to the cytoplasm might also be accounted for by the physical properties of RNPs, in which components appear to exist in a condensed liquid phase [17], enabling a degree of deformability. Thus, flexibility of either NPC and/or granule structure allows some larger RNPs to pass through the NPC.

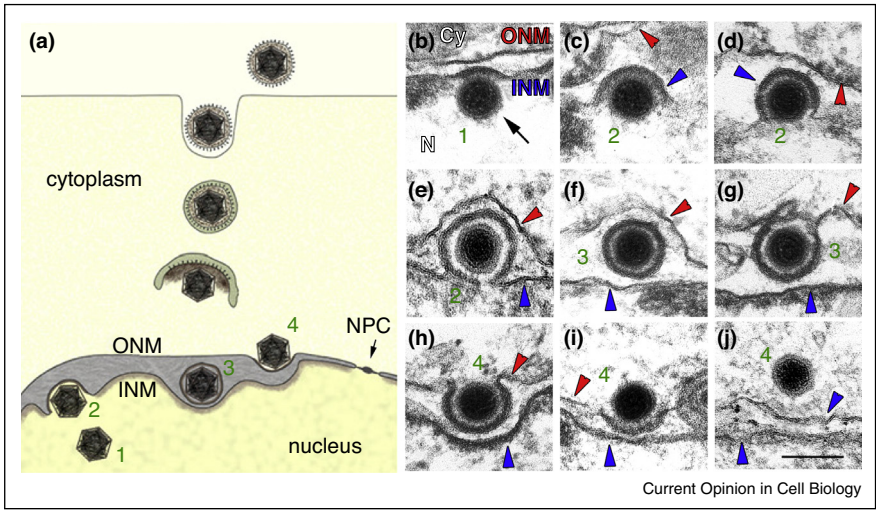
An alternative, NPC-independent mechanism for nuclear exit, has been uncovered from studies of the nuclear egress of Herpes viruses (HVs). HV nucleocapsids, themselves large nucleoproteins, are assembled in the nucleus and must escape to the cytoplasm for maturation and release from the cell. This mechanism, NE-budding, appears to be a conserved mechanism for the nuclear export of very large RNPs which are assembled in the nucleus.

HV egress from the nucleus

HVs are clinically-important double-stranded DNA viruses endemic in human and animal populations and induce infections such as oral and genital herpes caused by Herpes simplex, chicken pox and shingles, caused by Varicella zoster, mononucleosis, caused by Barr-Epstein virus, and Kaposi's sarcoma caused by Human herpesvirus 8. As is typical of viral replication, both viral and cellular proteins are employed through all phases of infection, replication, maturation and shedding from the host cell. Given the large size of HV nucleocapsids (~125 nm) they escape from the nucleus though a process of NE membrane remodeling, involving envelopment and de-envelopment of the nucleocapsid at the NE [18,19,20,21,22,23,24]. A widely accepted model of this process is that nucleocapsids disrupt the nuclear lamina, gaining access to the INM. Subsequently, they undergo primary envelopment by deforming the INM, thus budding into the perinuclear space (between INM and ONM; Figure 1a). In this way, perinuclear nucleocapsids become enveloped by an INM coat. During de-envelopment, the capsid INM coat fuses with the ONM, liberating a naked capsid into the cytoplasm (Figure 1a) for secondary envelopment at the Golgi and release from the cell.

The so-called Nuclear Egress Complex (NEC), required for primary envelopment at the INM, has been well characterized [20,22,23,24,25,26]. For simplicity, here we use the Herpes Simplex Virus-1 (HSV-1) protein nomenclature, although similar viruses encode orthologs which may bear different names. The HSV-1 NEC requires a complex of two virally-encoded proteins,

Figure 1



Nuclear egress of HV nucleocapsids. A diagrammatic representation of the envelopment and de-envelopment process at the NE **(a)**. Secondary envelopment of capsids in the cytoplasm and release of mature viral particles are also shown. Transmission electron micrographs of different stages of HV nuclear egress, showing nucleocapsid docking **(b)**, primary envelopment at the INM **(c,d,e)**, residence at the perinuclear space **(f)**, de-envelopment at the ONM **(g-i)** and escape to the cytoplasm **(j)**. Panels **a** and **b-j** are from Figure 1c and Figure 2, respectively, from [21] and are used with permission. The numbers shown in **(b-j)** correspond to the stages similarly numbered in (a).