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Forced entry into the nucleus

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Abstract

Transport of macromolecules into and out of the nucleus is regulated by the nuclear pore complex (NPC). A new study[1] shows that mechanical force applied on the nucleus affects the transport rates across the NPC's diffusion barrier, modulating the nuclear localization of certain cargos, including some transcriptional regulators. These results suggest that mechanosensing by the NPC could be a general mechanism for triggering signaling pathways in response to mechanical force exerted on the cell.

A fundamental property of cells is the ability to detect and respond to environmental cues. Signaling pathways initiated by these cues oftentimes result in the alteration of gene expression patterns. In eukaryotic cells, the nucleus provides an opportunity for controlling gene expression through selective import or export of transcriptional regulators. The conduit for nucleocytoplasmic trafficking of molecules are pores that arise from the circumscribed fusion of the two lipid bilayer membranes of the nuclear envelope. These nuclear pores are gated by the nuclear pore complex (NPC), an ~110 MDa, ~1,000-protein assembly with a central transport channel that mediates both passive and active bidirectional transport of folded macromolecular cargo between the nuclear and cytoplasmic compartments[2]. The NPC positions natively unfolded protein regions that contain repeating phenylalanine-glycine (FG) motifs into its central transport channel to establish a diffusion barrier. Through self-associating interactions, FG repeats establish a mesh-like phase-separated compartment that limits the diffusion rates of transiting molecules in a size-dependent manner (Figure 1A). Whereas small cargos, typically ~40 kDa or less, can passively overcome the diffusion barrier, larger cargos must associate with mobile transport factors – importins and exportins, collectively termed karyopherins – whose binding affinity for FG repeats and ultrafast exchange kinetics allow for efficient ferrying across the diffusion barrier. The directionality of active transport is established by the small GTPase Ran, which exists in two distinct nucleotide-bound states. Inside the nucleus, Ran is maintained in its GTP-bound state, which both disassembles importin•cargo complexes and participates in the formation of exportin•Ran(GTP)•cargo complexes. In the cytoplasm, Ran's GTPase activity is stimulated to break exportin•Ran(GTP)•cargo complexes apart and, conversely, prevent the disassembly

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Competing interests

The authors declare no competing interests.

of importin•cargo complexes. Due to Ran's GTP-hydrolyzing activity, karyopherin-mediated active transport is an energy-dependent process.

Cryo-electron tomographic (cryo-ET) reconstructions of intact human NPCs, interpretable by docking crystal structures of its many nucleoporin protein subunits into the cryo-ET density, were initially obtained by imaging purified nuclear envelope fractions (Figure 1B)[3,4]. Architecturally, the NPC is composed of a symmetric core that consists of an inner ring harboring the central transport channel and two outer rings sitting on top of either side the nuclear envelope. Asymmetric components that provide key binding sites for transport factor complexes are attached to the outer rings and project into the nucleus and the cytoplasm[5,6]. The central transport channels of these early cryo-ET reconstructions possessed a diameter of ~45 nm. However, subsequent cryo-ET reconstructions of human NPCs, imaged *in situ* by focused ion beam (FIB)-milling whole cell specimens, display larger ~65 nm central transport channel diameters, establishing that the human NPC's inner ring is capable of dilating by at least ~20 nm[7,8]. Most recently, structural advances uncovered that the cohesion of the structured inner ring nucleoporins is established by unstructured linkers, conferring plasticity to the inner ring's eight spoke-like segments[6,9]. This evolutionarily conserved architectural feature allows large-scale subunit displacements and the reversible formation of lateral channels upon the central transport channel's constriction and dilation, as captured by cryo-ET imaging of fission yeast cells under hyperosmotic shock or energy depletion conditions[10]. Thus, membrane tension of the nuclear envelope, caused by various intra- and extra-cellular forces, directly affects the dilation state of the NPC's central transport channel. The realization that NPCs can undergo such massive conformational rearrangements in response to mechanical force raises a fundamental question: Could the cell utilize the NPC's central transport channel dilation to regulate nucleocytoplasmic transport?

In this issue of *Nature Cell Biology*, Andreu, Granero-Moya *et al.* explore the molecular mechanism by which force exerted on nuclei can control the nuclear localization of certain proteins. This study builds upon previous work by the same group, which demonstrated that mechanical force applied to nuclei of cultured mammalian cells results in nuclear accumulation of the transcriptional regulator YAP[11]. First, the authors applied mechanical force on nuclei either via the cantilever of an atomic force microscope, or by culturing adherent mammalian cells on polyacrylamide substrates of different stiffness. Nuclei are anchored by the linker of nucleoskeleton and cytoskeleton (LINC) complex, which spans the nuclear envelope to connect the nuclear lamina to the cell's cytoskeleton. Extra-nuclear forces, including constitutively generated actomyosin strain, are translated into nuclear envelope membrane tension by the LINC complex, as elegantly shown by employing a dominant negative mutation in the LINC complex component nesprin to mechanically decouple the nucleus from the cytoskeleton. Next, the authors analyzed the effect of applying mechanical force on cells by measuring rates of nuclear influx and efflux in live cell nuclei by fluorescence photobleaching methods or by tracking cargos with engineered photoactivatable nuclear localization signals (NLSs, recognized by importins) and nuclear export signals (NESs, recognized by exportins). Remarkably, what they found was that force exertion on nuclei increased the rate of passive diffusion for small cargos, without significantly affecting the size selectivity of the barrier. Moreover, the same treatment also

increased the transit rate for importin-β, the karyopherin transport factor that shuttles cargo into the NPC thanks to the cargo unloading performed by exclusively nuclear Ran(GTP). The differential effect on the passive and active transport rates of certain cargos led the authors to reason that exerting force on the nucleus could be exploited by the cell to modulate steady-state nucleocytoplasmic cargo localization. For small macromolecules or macromolecules that are not recognized by karyopherins, transport across the nuclear envelope is driven by passive diffusion. On the contrary, the localization of very large cargos or cargos with high affinity NLSs or NESs is determined by active transport, regardless of the dilation state of the NPC. However, the authors found that there is a "sweet spot" for cargos of moderate size and affinity for karyopherins, for which the diffusion barrier represents a hurdle and whose active transport benefits from the increased rates of karyopherin shuttling that occur when force is applied on the nucleus. Whereas those cargos' localization may be driven by passive diffusion under low-force conditions, it is active transport that dominates under high-force conditions. At last, the authors translated this novel conceptual understanding into a mathematical model, which was remarkably predictive of the nucleocytoplasmic transport mechanosensitivity for engineered model cargos of varying size and karyopherin affinity, as well as for prominent transcriptional regulators Twist1, Snail, SMAD3, GATA2, and NF-κB. The finding that the NPC's mechanosensitivity can modulate the nucleocytoplasmic transport of cargos with a certain size and affinity for karyopherins generates exciting prospects for the discovery of novel mechanotransduction pathways. Yet, it also raises questions about how cells that are exposed to persistent mechanical perturbation, such as pulsating cardiomyocytes, epithelial cells exposed to changing osmotic pressures, or extravasating metastatic cancer cells, deal with the challenge of regulating nucleocytoplasmic transport under these conditions.

Andreu, Granero-Moya *et al.*'s results indicate that the dilation state of the NPC's central transport channel affects the performance of the diffusion barrier. By extension, this establishes the functional importance of the dilated and constricted NPC states observed in cryo-ET reconstructions for the mechanosensitive regulation of nucleocytoplasmic transport. Future studies to determine the biophysical basis for this phenomenon are eagerly awaited.

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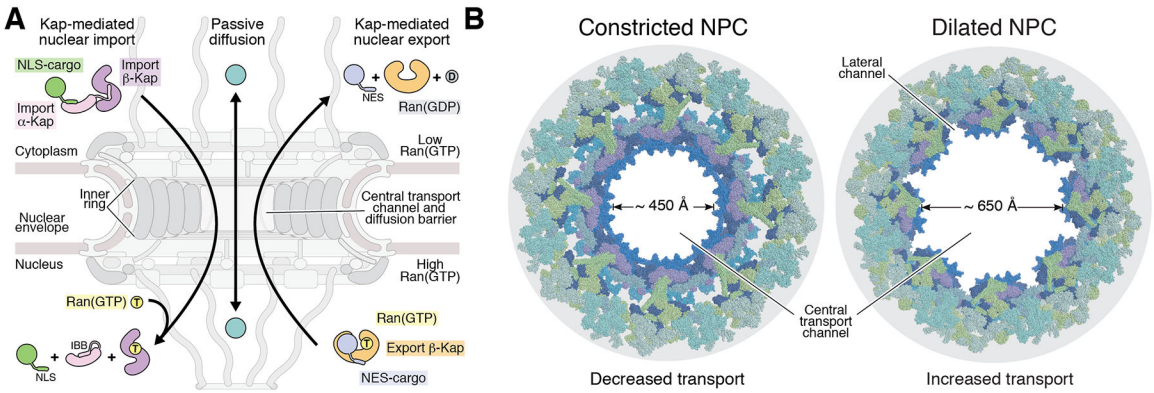

Fig. 1 |. Mechanical tuning of nucleocytoplasmic transport.

(**A**) Active karyopherin (Kap)-mediated and passive transport pathways through the central transport channel of the nuclear pore complex (NPC). In the classical import pathway, the cargo's NLS binds to an adaptor α-Kap (importin-α), displacing its autoinhibitory importin-β-binding (IBB) α-helix, which binds to an import β-Kap (importin-β); the ternary import complex shuttles across the diffusion barrier and disassembles upon arrival in the nucleus by Ran(GTP) binding. In the export pathway, NES-presenting cargo forms a ternary complex with Ran(GTP) and an export β-Kap (exportin) that shuttles across the diffusion barrier; in the cytoplasm, Ran's GTPase activity is stimulated to disassemble the complex. (**B**) Cryo-ET reconstructions of the constricted and dilated human NPCs interpreted by docking crystal and single particle cryo-EM structures of its nucleoporin protein subunits[5,6,9].