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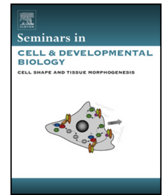

Review

Biomechanics of the transport barrier in the nuclear pore complex

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ABSTRACT

The nuclear pore complex (NPC) is the selective gateway through which all molecules must pass when entering or exiting the nucleus. It is a cog in the gene expression pathway, an entrance to the nucleus exploited by viruses, and a highly-tuned nanoscale filter. The NPC is a large proteinaceous assembly with a central lumen occluded by natively disordered proteins, known as FG-nucleoporins (or FG-nups). These FG-nups, along with a family of soluble proteins known as nuclear transport receptors (NTRs), form the selective transport barrier. Although much is known about the transport cycle and the necessity of NTRs for chaperoning cargo molecules through the NPC, the mechanism by which NTRs and NTR•cargo complexes translocate the selective transport barrier is not well understood. How can disordered FG-nups and soluble NTRs form a transport barrier that is selective, ATP-free, and fast? In this work, we review various mechanical approaches – both experimental and theoretical/computational – employed to better understand the morphology of the FG-nups, and their role in nucleocytoplasmic transport. Recent experiments on FG-nups tethered to planar surfaces, coupled with quantitative modelling work suggests that FG-nup morphologies are the result of a finely balanced system with significant contributions from FG-nup cohesiveness and entropic repulsion, and from NTR•FG-nup binding avidity; whilst AFM experiments on intact NPCs suggest that the FG-nups are sufficiently cohesive to form condensates in the centre of the NPC lumen, which may transiently dissolve to facilitate the transport of larger cargoes.

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Contents

1. Introduction 42
   1.1. Structure of the NPC 42
   1.2. Intrinsically disordered FG-nups 43
   1.3. Nucleocytoplasmic transport 43
   1.4. Nuclear transport receptors 43
   1.5. Probing the NPC with atomic force microscopy 44
2. Mechanics of the transport barrier 45
   2.1. Hypotheses for the mechanisms of transport 45
   2.2. Polymer mechanics of FG-nups grafted in the planar geometry 46
   2.3. Probing the FG-nups of intact NPCs 49
3. Concluding remarks 49
   Acknowledgements 50
   References 50

1. Introduction

1.1. Structure of the NPC

The nuclear pore complex (NPC) is a selective gateway for all macromolecules entering or exiting the nucleus. Small molecules can passively diffuse through the NPC (diameter $\lesssim$5 nm), but larger

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molecules must bind to small chaperone proteins, called nuclear transport factors (NTRs), in order to translocate the NPC.

The NPC is a supramolecular, proteinaceous assembly, comprised of ~30 different nuclear pore proteins (hereby termed 'nucleoporins', or 'nups'), which assemble to form a pore across the nuclear envelope [1]. Each nucleoporin is present in many copies, to give a total of ~1000 nups, with a combined mass of ~60–125 MDa per pore [1–3]. The NPC has an eight-fold rotational symmetry around its central axis. At the cytoplasmic face of the NPC, eight filaments protrude into the cytoplasm; and at the nucleoplasmic face, a basket structure, also attached by protein filaments, extends into the nucleoplasm [4]. The NPC is modular: structural nucleoporins interact to form larger nup-subcomplexes, which in turn form the scaffold of the NPC. The structural scaffold of the NPC is made of three distinct rings: the cytoplasmic ring complex (CRC), the nucleoplasmic ring complex (NRC), and the inner ring complex (IRC) [3,5–10]. Both the CRC and the NRC are themselves made from two reticulated ring structures, comprised of 16 copies of the Y-shaped Nup107 subcomplex (in the case of the human NPC, Nup107 is formed from 10 nucleoporins [3]) [11]. These reticulated ring structures are then interlaced with other nups, and nup-subcomplexes, conferring structural heterogeneity between the CRC and the NRC. The IRC, although comprised of different nup-subcomplexes (Nup93 and Nup62 [12,13]), has a remarkably similar morphology to the CRC and NRC [14,15]. The Nup93 and Nup62 subcomplexes form Y-shaped assemblies – akin to the Nup107 subcomplex – which intercalate to form a ring. The IRC then connects with the two outer rings (the CRC and NRC) via Nup155: a component of the Nup93 subcomplex [14,15].

Of the ~30 nucleoporins that form the NPC, only about half are structural: forming the Y-shaped subcomplexes and other nup-subcomplexes intercalated into the outer and inner rings [14]. Anchored to the inner wall of the central channel of the NPC are many unstructured and intrinsically disordered nucleoporins. These disordered nups contain hydrophobic sequences rich in phenylalanine-glycine (FG) repeats (hereby termed FG-nups: this term incorporates all hydrophobic sequences – such as FG, FxFG, and GLFG, amongst others – found in the disordered nups [16]). These are further discussed in section 1.2. From their anchoring sites at the inner wall of the NPC, the FG-nups emanate into the central channel and form the selective barrier to transport (see Fig. 1a for a schematic of the NPC).

Although the nucleoporins between species can be very divergent (e.g., between the structurally integral Nup107 subcomplex in yeast and humans [3,17]), the overall architecture is conserved across all metazoans [18–21]. However, even with high-resolution data obtained for the scaffold structures of yeast, human, and *Xenopus laevis* NPCs [3,10,22], the disordered nature of the FG-nups in the central channel means they have eluded the various structure-determination methods that depend on averaging techniques. In order to understand what morphology these FG-nups assume in the NPC, and how they can form a selective barrier to nucleocytoplasmic transport, other methods are required.

1.2. Intrinsically disordered FG-nups

The morphology of FG-nups in the central channel is of great importance for understanding the mechanism of selective transport through the NPC, and as such has been a topic of debate for many years [23–29]. The FG-nups occluding the central transport channel of the NPC are natively disordered [16,30,31]. As mentioned above, they contain repeating sequences of hydrophobic amino acids (such as FG, FxFG, FxFx, PSFG, and GLFG [16]), through which they can interact with one another, affording them a certain 'cohesiveness' [32] – with more cohesive FG-nups forming more compact morphologies, and less cohesive FG-nups forming more extended morphologies [26,32]. However, the conformation of FG-nups is not solely dependent upon the hydrophobic interactions between FG-domains. Repulsive, charged amino acids in the spacer regions of the FG-domains can counteract the cohesive interactions of the hydrophobic sequences [26]. A higher ratio of charged to hydrophobic amino acids in the FG-domain decreases cohesion and leads to protein extension; whilst a lower ratio affords increased cohesion and greater FG-nup compaction. It is probable that this heterogeneity in cohesion, compaction, and extension between the different FG-nups is used to nuance the NPC function along its axis of transport. For example, the glycosylated human Nup98, which is sufficiently cohesive to form hydrogels in vitro, and is found anchored to the inner ring of the NPC [12], may, in the confines of the cylindrical pore geometry, interact with FG-nups with diametrically opposed anchoring site, to form a size-excluding meshwork – thus creating a selectivity barrier to transport. The human Nup153 however, located nearer the nuclear periphery [12], may, as well as comprising part of the transport barrier, act as a nucleation site for proteins required for the active transport of macromolecules [33]. However, the impact of FG-nup heterogeneity on the global morphology of FG-nups in the selective transport barrier deep inside the central channel is not well understood.

1.3. Nucleocytoplasmic transport

Although the NPC is the gateway through which all molecules must pass during nucleocytoplasmic transport, it is not the sole participant in the filtering process. The translocation of large molecules (diameter $\gtrsim$5 nm, or mass $\gtrsim$40 kDa) is facilitated by complexation with other smaller proteins, called nuclear transport receptors (NTRs – also termed karyopherins, importins, exportins, and transportins). Nuclear transport receptors recognise and bind specific sequences of basic amino acids on the cargo proteins. These sequences are called nuclear localisation sequences (NLSs; for import) or nuclear export sequences (NESs; for export). Once bound, the affinity of the NTRs for the FG-nups in the NPC enables transport of the NTR•cargo complexes, via a mechanism that is not yet fully understood.

Furthermore, in this system a bias is required to ensure that once an NTR•cargo complex has completed a transport event, it does not (at least on average) re-enter the NPC and reverse its journey. This is accomplished by the 'Ran system'. At the end of an import event, a RanGTP molecule binds to the NTR of an NTR•cargo complex, inducing a conformational change and displacing the cargo molecule, thus releasing both the cargo molecule and the newly formed RanGTP•NTR complex into the nucleoplasm. This displacement reaction probably takes place whilst the NTR•cargo complex is interacting with Nup153 at the nuclear periphery of the NPC [33]. The RanGTP•NTR complex can then either return to the cytoplasm through the NPC, or bind to the nuclear export sequence of another cargo molecule, to facilitate its exit from the nucleus. Once in the cytoplasm, GTP hydrolysis of Ran by RanGAP and RanBP1 frees the cargo molecule and the NTR, leaving the hydrolysed RanGDP. This RanGDP:RanGTP gradient – with more RanGDP in the cytoplasm and more RanGTP in the nucleoplasm – is maintained by the nuclear RanGTPase RCC1, and is integral for maintaining directionality of transport across the NPC [35].

1.4. Nuclear transport receptors

NTRs belong to a family of proteins with approximately 20 members that all share similar properties with the prototype importin-β (termed Impβ): i.e., they can bind to RanGTP at the N-terminus, they are hydrophobic, and they exhibit an affinity for the FG-nups [36]. Impβ is a ~100 kDa protein with ~19 HEAT-repeat sequences (i.e., antiparallel α-helical domains) that interact with

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