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Review

Floppy but not sloppy: Interaction mechanism of FG-nucleoporins and nuclear transport receptors

Iker Valle Aramburu, Edward A. Lemke*

Structural and Computational Biology Unit and Cell Biology and Biophysics Unit, Meyerhofstrasse 1, 69117 Heidelberg, Germany

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ABSTRACT

The nuclear pore complex (NPC) forms a permeability barrier between the nucleus and the cytoplasm. Molecules that are able to cross this permeability barrier encounter different disordered phenylalanine glycine rich nucleoporins (FG-Nups) that act as a molecular filter and regulate the selective NPC crossing of biomolecules. In this review, we provide a current overview regarding the interaction mechanism between FG-Nups and the carrier molecules that recognize and enable the transport of cargoes through the NPC aiming to understand the general molecular mechanisms that facilitate the nucleocytoplasmic transport.

Contents

1. Introduction to the nucleocytoplasmic transport and the transport paradox

The exchange of biomolecules between the nucleus and the cytoplasm takes place through the nuclear pore complex (NPC). It is typically considered that molecules below 40 kDa are able to freely diffuse through the NPC. Molecules above this molecular weight require the interaction with nuclear transport receptors (NTRs) to successfully cross through the NPC (see review in Ref. [1]). However, this size limit appears to be rather a gradient, than a strict cut-off limit [2,3]. NTRs, independent of being loaded with a cargo, are able to cross the permeability barrier of the NPC which is primarily formed by a group of proteins called FG-nucleoporins (FG-Nups). NPC crossing is an energy independent process but the directionality of the molecules being actively transported by NTRs depends on a RanGTP/GDP gradient formed between the nucleus and the cytoplasm [4]. RanGTP is more abundant in the nucleus, and can bind to import complexes, causing the cargo release in the nucleoplasm. Vice versa, when bound to export NTRs, RanGTP increases the affinity for export cargoes. The exported cargo can then be released from the export complex by RanGTP hydrolysis in the cytoplasm. The high concentration of RanGTP in the nucleus and of RanGDP in the cytoplasm is caused by the nuclear localization of RanGEF and RCC1, that exchange GDP for GTP, and the cytoplasmic localization of RanGAP, which hydrolyzes RanGTP into RanGDP. Cytoplasmic RanGDP is then recycled back to the nucleus by the NTR NTF2 (see review of RanGTP/GDP cycle in Ref. [5,6]).

The crossing of the NPC by NTRs with or without cargoes is a fast and selective process. Single molecule fluorescence microscopy

* Corresponding author.
E-mail address: lemke@embl.de (E.A. Lemke).

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experiments have enabled the measurement of the translocation time through the NPC of different cargoes by following the trajectories of labeled molecules (reviewed in Ref [7]). In this way, it was directly measured that molecules of different sizes can cross the NPC on millisecond timescales [8,9]. During this time, the NTRs interact with phenylalanine glycine motifs (FG-motifs) from the FG-Nups, on their way throughout the NPC barrier, undergoing multiple binding and unbinding events.

Using the latest published structure of the NPC [10], we can estimate the dimension of the NPC that is occupied by the FG-Nups of the central channel (Fig. 1A). We approximately measured a radius of 22.5 nm and a height of 24 nm for the inner ring of the NPC resulting in a calculated volume of around 38200 nm^3. Then we calculate the number of FG, GF and F residues, which are motifs or residues that could engage in the binding to NTRs, present in the disordered region of the FG-Nups based on the known stoichiometry at the NPC [11]. The estimated concentration of FGs at the central NPC barrier is ~160 mM. If we then consider in addition the GF residues (~80 mM) we would have a local concentration of binding sites of ~240 mM which increases up to ~260 mM if we consider the F residues only. Moreover, different *in vitro* equilibrium studies of FG-Nups and NTRs have reported high binding affinities from the nM the μM range [12–16]. As high affinity values are often associated with tight specific long-living complexes, it seems as a paradox that NTRs are not mainly stuck to the surface of the FG-Nup barrier, but can actually migrate through it very fast.

To further understand the nucleocytoplasmic transport, it is key to study the interaction mechanism between FG-Nups and NTRs. In this review, we provide an overview of the characteristic features of FG-Nups and NTRs with a focus on the latest studies on the interaction between FG-Nups and NTRs. This brings us closer to elucidating the nucleocytoplasmic transport process and to answer how fast and specific transport could be achieved. We will argue that this fundamental concept is largely robust and independent of what "model" one assumes for the structure of the actual, so far elusive permeability barrier.

2. Characteristic features of FG-nucleoporins and nuclear transport receptors

2.1. FG-nucleoporins, the molecular doorkeepers of the NPC

FG-Nups line the central channel of the NPC (Fig. 1B) and are composed of at least a folded domain, involved in anchoring the protein to the NPC, and an intrinsically disordered domain (IDD) protruding into the channel [17]. IDDs or whole intrinsically disordered proteins (IDPs, here for simplicity all called IDP) are proteins that lack a stable secondary and tertiary structure and are disordered under native conditions [18,19]. Typically, these structural features are associated with a high net charge and a low hydrophobicity, which allows them to remain unfolded in solution under physiological conditions [19]. FG-Nups however, contain on average high mean hydrophobicity, which is comparable to the levels of hydrophobicity present in globular proteins. FG-Nups also contain a very low net charge, which is lower than in typical disordered domains [20,21]. Furthermore, FG-Nups contain multiple F residues across their sequence, many of which are in direct neighborhood to a G (Fig. 1C) [17,22]. Based on the different amino acids flanking the F residues different types of FG-motifs have been classified: FxFG, GLFG, PxFG, SxFG and FG are the most frequently seen FG-motifs but less abundant FG-motifs have also been identified in some FG-Nups [21,23,24]. In many cases, however, it still remains elusive to what extentthe nature of those repeats directly correlates to a specific function.

The primary sequence of the IDP region of FG-Nups does not have a high sequence conservation, which is often the case for disordered proteins that show a high amino acid substitution rate [25]. A bioinformatic study of FG-Nup sequences showed that the intrinsic disorder of FG-Nups is a conserved feature. However, FG-Nups also showed rapid evolution rates compared to other proteins. Another feature that seems to be conserved across FG-Nups is the hydrophilic inter-motif sequences of 10–20 amino acids present between the FG-motifs [23]. Those "linkers" also show a high amino acid substitution rate but maintain hydrophilic physicochemical properties [23].

The physicochemical properties of FG-Nups are directly related to a topic of central relevance to the field, i.e. how the actual permeability barrier is formed. The structure of the permeability barrier, which depends in part on the general question if FG-Nups interact with each other (are cohesive) or to what degree they rather repel each other (which could yield to brush type structures), still hasn't converged to a single model that the whole field can easily agree on [17,26–33]. At the core of this problem is that the study of the barrier *in situ* is extremely challenging because it is a dynamic structure with a size below the resolution limit for conventional light microscopy. Several high resolution cryo-electron tomography 3D maps of the NPC have meanwhile been presented [10,34]. While most folded proteins can already be fitted into the maps with secondary structure resolution, the actual transport conduit shows up as a large empty channel, because the dynamic structures are averaged out and remain invisible to electron tomography technology. In light of a lack of technology to directly visualize the permeability barrier and the inability to purify entire functional NPCs, scientists have designed various elegant approaches to study how the permeability barrier might be formed *in vivo*. FG-Nups have shown to be able to adapt several single and macromolecular states, ranging from collapsed to extended states as single proteins in solution, as well as brushes and films when mounted on surfaces [26,28,35–37]. In addition, FG-Nups can undergo transitions to supramolecular assemblies giving rise to tough hydrogels, as well as amyloid fibers [21,38,39]. Based on these different studies a large set of models have been developed. In this review, we will not get into the discussion of these models, and rather point the reader to interesting reviews on this topic [20,40,41]. Instead, we will focus on the one thing that most models agree on: The required interaction between NTRs and FG-Nups to cross the selective barrier of the NPC and the roughly known concentration of FGs in the permeability barrier. We will first describe the structural features of different NTRs that are required for the successful NPC crossing followed by an overview of what we learned from recent structural and biochemical studies about the interaction between FG-Nups and NTRs.

2.2. Structural features required for NPC crossing

Binding to NTRs is the major route for cargoes to get across the NPC. To do so, NTRs can recognize and bind nuclear localization and nuclear export signals (NLS and NES respectively) from different cargoes. Many of these carrier proteins belong to the Importin-β/β-karyopherin superfamily (for review see Refs. [42,43]). Members of this superfamily contain an overall high structural similarity but low, between 15 and 20%, sequence identity [44]. β-karyopherins like the import receptor Importin-β and Transportin or the export receptor CRM1 are composed of a tandem series of HEAT repeats (reviewed in Ref. [45]). HEAT is an acronym that comes from the different proteins where this motif was originally found: Huntingtin, elongation factor 3, the A subunit of the protein phosphatase 2A and the kinase TOR1. Each HEAT motif is composed of approximately ~30-40 amino acids that form two amphiphilic α-helices (A-helix and B-helix) (Fig. 1D) with one side containing hydrophobic residues and the other side being enriched in hydrophilic amino