Nucleocytoplasmic transport in yeast: a few roles for many actors

Jindriska Fiserova and Martin W. Goldberg

School of Biological and Biomedical Sciences, Durham University, Sciences Laboratories, South Road, Durham DH1 3LE, U.K.

Abstract

Eukaryotic cells have developed a series of highly controlled processes of transport between the nucleus and cytoplasm. The present review focuses on the latest advances in our understanding of nucleocytoplasmic exchange of molecules in yeast, a widely studied model organism in the field. It concentrates on the role of individual proteins such as nucleoporins and karyopherins in the translocation process and relates this to how the organization of the nuclear pore complex effectively facilitates the bidirectional transport between the two compartments.

Introduction

Eukaryotic cells have developed a series of highly controlled processes of transport between the nucleus and cytoplasm. As with any controlled exchange, it comprises guarded gates, goods to be exchanged, which are provided with identification codes, and a shipping service. Within the NE (nuclear envelope), NPCs (nuclear pore complexes) serve as multiprotein gates that facilitate controlled exchange of macromolecules and ions between the nucleus and the cytoplasm with the assistance of Kaps (karyopherins) as carriers. The identification of cargo to be transported is via its specific NLS (nuclear localization sequence) or NES (nuclear export sequence). Apparently, all components of the system evolved hand in hand to fulfill the demand for speed and specificity inside the constantly changing environment of the cell. Despite significant progress towards our understanding of the transport processes or at least partial details of each of the transport components, after almost half a century of intense research, it remains a challenge to combine this knowledge into a clear picture. The present review focuses on the latest advances in nucleocytoplasmic transport with emphasis on the yeast model to provide an up-to-date picture of the process.

Translocation through the NPC

NPCs of eukaryotic cells share a general architecture. A cylindrical central channel perpendicularly transverses the NE between cytoplasmic and nucleoplasmic rings. The two respective rings anchor peripheral structures, nuclear basket and cytoplasmic filaments that emanate outwards from the NPC ([1–3]; reviewed in [4]). When viewed en face, circular rings of eight round subunits are observed from the cytoplasmic side, whereas cage-like structures can be seen from the nucleoplasmic side [1,5]. Finally, within the central channel there is often present a particle of varying size and uncertain origin [1,3,6,7]. Biochemically, NPCs are thought to be composed of multiple copies of only approx. 30 proteins, Nups (nucleoporins) [8,9]. These can be grouped with respect to their structure, dynamics, putative localization or role within the NPC [9–11].

A common feature of the models addressing the translocation event is the emphasis on the role of repetitive domains of phenylalanine and glycine (FG domains) that are part of approx. one-third of Nups and create up to 10% of NPC mass [9]. The FG domains are believed to adopt no secondary structure [12] and so are unfolded and highly dynamic, locating inside the central channel and its immediate vicinity [13,14]. Different models vary in predicting the actual behavior of FG domains, as well as the nature of the diffusion barrier, the means of overcoming this barrier as well as translocation through it (reviewed in [15]). The selective phase model [16] proposes very weak hydrophobic interactions between FG domains that would result in a sieve-like structure of the central channel. To overcome this physical barrier requires binding of the transport complex to the FG domains and ‘melting through’ the hydrogel structure in the central channel [17]. The Brownian affinity model [8], on the other hand, stresses the role of peripheral structures as docking sites for cargo to help overcome the energetic barrier of the unstructured FG domains. Initial interactions between cargo and FG domains would be crucial for translocation initiation, continued with ‘Brownian-like’ movement through the NPC. Both models find support in in vitro experiments [8,14,18,19], while in vivo experiments provide rather ambivalent data [20–22]. Other models that address the mechanism of transport include the affinity gradient model [23], the oily spaghetti model [24] or the two-gate model [19].

The role of FG domains in the translocation process was approached in a large screen of FG domain deletion mutants. Surprisingly, over half of the total mass of FG domains can...
be deleted without strongly affecting the permeability barrier of the NPC or loss of viability in yeast [20]. Such a result is contradictory to the selective phase model, in which the actual number of FG repeats determines the restrictive size of the diffusion barrier. Interestingly, when different combinations of FG domains were deleted, there was a variable effect on yeast viability and different transport pathways were affected [20,22]. Thus the studies suggested the existence of functionally independent translocation pathways for different transport receptors. Additionally, deletion of all 'asymmetric' FG domains (presumed to locate to peripheral structures such as the basket and cytoplasmic filaments) had little effect on cell viability, while deletion of certain combinations of 'symmetrically' localized FG domains turned out to have severe effects or be lethal [20]. Moreover, in yeast cells where the 'asymmetric' FG domains were swapped between different Nups, there were no obvious transport defects [21]. This implies either that 'asymmetric' FG domains are dispensable for transport and non-FG domains have a role in cargo docking or that symmetric FG domains may extend into the cytoplasmic area, or, finally, that it is a necessity to reconsider the Brownian affinity model. Supporting evidence for the former might be the finding that non-FG Nup53 can serve as a specific docking site [25]. The attempt to reconsider the model in response to deletion studies was also made and a related model called 'reduction of dimensionality' was proposed [26]. In this model, FG domains were proposed to line the inner channel to create a coherent surface. This surface would have three different sections: (i) an initial binding site for cargo, (ii) sliding of the cargo-bound transport factor along the FG surface of the channel by a two-dimensional walk and (iii) cargo release. This model incorporates a selectivity filter to restrict diffusion of large particles in a similar way to the Brownian affinity model (the narrow central channel restricts the access of large molecules that cannot bind). It accounts for FG-deletion mutant studies because the number of FG domains would not be critical and the centrally located ones would be more important for the actual translocation process. Lastly, it was also supported in transposition experiments using optical single transporter recording, which combines fluorescence detection, photochemical detection and membrane patching to monitor both the passive and the active transport through a single NPC [27,28].

'Symmetric' or 'asymmetric' positions of individual Nups within NPCs in yeast were originally addressed by Rout et al. [8]. That study showed a varying distribution of Protein A tags on individual Nups within NPCs by immunogold electron microscopy. However, the detected distribution of the C-terminally localized Protein A tags does not necessarily overlap the distribution of their mostly N-terminally localized flexible FG domains that could stretch out much further. A few other attempts to localize Nup distribution in yeast involve an SEM (scanning electron microscopy) study of Kiseleva et al. [5] that showed the cytoplasmic position of Nup159 and Nup116. Given that SEM is used to image surface structures, the localization of Nup159 and Nup116 more deeply inside the NPC structure cannot be excluded.

Our TEM (transmission electron microscopy) immunolocalization studies indeed confirm that the distribution of FG domains of Nup116 varied in individual NPCs and could be detected as being (i) strictly nuclear, (ii) cytoplasmic, (iii) evenly distributed or (iv) slightly biased towards either side of the NPC (J. Fiserova, unpublished work). These observations probably reflect the flexibility of FG domains and stress the individual character of NPCs. Importantly, it also shows that FG domain localizations must be extrapolated carefully from immunoelectron microscopy studies.

Deletion of certain Nups often resulted in NPC aberrations such as blebbing, membrane herniations or swelling [29–31]. Deletion of other Nups (Nup82), on the other hand, had no direct effect on NPC structure, NPC number or clustering [32]. Interestingly, deletion of just FG domains is sufficient to cause membrane aberrations or swelling (J. Fiserova, unpublished work), which either implies that the import of proteins necessary to maintain the NE was disrupted or points to other yet unspecified roles of FG-Nups in NE structural determination and maintenance.

Karyopherins mediate the translocation event

The movement of proteins and RNA between the nucleus and cytoplasm is facilitated by a group of soluble proteins called Karyopherins. These proteins recognize NLSs or NESs of their cargoes and 'deliver the goods' across the NPC. The karyopherin β family counts for most of the protein and RNA transport events [33]. There are 14 members of the Kap β family identified in yeast, which mediate either import into or export out of the nucleus. Only one yeast Kap (Kap142/Msn5) is capable of mediating both import and export [34]. Studies of the mammalian homologue of Kap β showed that the protein can adopt different conformations in response to binding to different cargoes (reviewed in [35]) and suggested a putative level of transport regulation by altering the Kap β stereological features. Not only can Kaps recognize more than one cargo and potentially also more than one NLS, but also vice versa, cargo can be recognized by more than one Kap [36]. For instance, yeast Kap121 recognizes NLSs with lysine-rich residues as well as a unique class of NLSs with an abundance of arginine and glycine residues, rg-NLS [37]. Proteins with the rg-NLS can be also imported by Kap104, which can compensate for Kap121 when needed [37]. Similarly, Kap121, Kap108 and Kap119 can supplement for the absence of Kap123 [38]; however, complete loss of Kap123 results in growth defect [39], suggesting decreased rates of transport in the absence of Kap123. Kap114 is equipped with multiple cargo-binding domains and is thus able to import several proteins simultaneously [40]. Additionally, nuclear import of histones is linked to various Kaps [41,42]. The ability of Kaps to act redundantly is best demonstrated by the fact that only four known β Kaps are essential in yeast (Kap95, Kap121, Crm1 and Cse1) [33]. Thus many proteins can use several biochemical routes into and out of the nucleus demonstrating flexibility of the transport mechanism to respond to changing needs of the cell.
How is the NPC organized structurally to facilitate diverse transport routes?

It is largely assumed that different biochemical routes are achieved by binding of the cargo to different Kaps, which in turn bind to different FG domains during translocation. The number and variability of FG domains allow karyopherins to use specific combinations on their way through the NPCs and ensure high saturation levels for transporting large amounts of cargo as well as redundancy to substitute for occasional defects [43]. Nevertheless, the structure of the central channel and the ‘central transporter’ [6,17] itself are surrounded by mystery. Several important aspects are not clear: (i) whether the routes through the channel are physically separated or overlapping; (ii) whether the entrance and exit represent common or overlapping platforms on peripheral structures or whether they are nearer to the NPC core; or (iii) even whether the diffusion of smaller molecules and ions takes place through the presumed ‘diffusion channels’ [17,44] localized on the channel periphery or directly through the channel centre.

The first yeast three-dimensional NPC model of Yang et al. [2] displayed a centrally located transporter, approx. 30 nm wide, in various gating conformations. It was assumed that translocation occurs within the transporter, whereas diffusion of the smaller molecules and ions takes place via diffusion channels located just outside the transporter. With the FG domain-based models of NPC transport emerging later on, the central transporter was replaced with a randomly organized network of unfolded FG domains [19]. The centrally located object in the NPC centre observed frequently by electron microscopy techniques became more often considered as cargo caught in transport [3,7]. Interestingly, a particle in the centre of the NPC appeared only when a mutant form of Importin β was added to block the channel in Xenopus oocytes [45], implying that observed ‘plugs’ might generally represent cargo that is being translocated rather than an intrinsic part of a channel. Another interpretation would be also plausible: when sealed by Importin β molecules, central transporter conformation might become more apparent when observed with atomic force microscopy. However, the question still remains as to how the FG domains are organized inside the channel to catalyse active bidirectional motion and passive diffusion simultaneously.

The specific biochemical interactions of Kaps–cargo with individual Nups over the translocation route using in vitro techniques can be more easily resolved than the physical translocation of the Kap–cargo along the NPC channel in vivo. Indeed, various attempts to map the transport routes and interactions of Kaps with the respective FG domains in vitro have been made. During translocation through the NPC, GLFG domains of Nup116 and Nup100 were found to serve as binding sites for Kap95 and Mex67 in yeast [46,47]. At the NPC periphery, Kap60, Kap95 and Kap123 interact with Nup1, Nup2 and Nup60 [48,49], which serve as docking and termination sites. Yeast lacking Nup60 exhibited defects in nuclear export of Kap60, as well as nuclear import of Kap95–Kap60-dependent cargoes, and diffusion of small proteins across the NPC [48,49]. The active role of Nup1 and Nup2 in Kap 95–Kap60 translocation was confirmed later [48,50]. Cells lacking Nup2 showed a specific defect in both Kap95 import and Kap60 export, indicating that Nup2 is required for efficient bidirectional transport across the NPC. In yeast, the essential Kap121 also interacts with Nup53, showing that non-FG Nups also play an important role in translocation events [25]. Interestingly, the interaction also functions in assembly of Nup53 into the NPC [43]. Using FRET (fluorescence resonance energy transfer), Damelin and Silver [51] showed that Kap121 interacts with the FG-Nups Nup116, Nup1 and Nup2, and with non-FG-Nups Nup133, Nup145, Nup188, Nup53 and Nic96. Kap42/Msn5, which is capable of bidirectional transport, shared most binding contacts with Kap121, suggesting overlapping pathways for the two Kaps or possibly even for export and import routes as well as co-ordinated action of FG- and non-FG-Nups during translocation events [51]. Non-FG-Nup82 was shown to be important for Kap142/Msn5-mediated nuclear protein export and Kap95-mediated protein import in synthetic lethal screens with Nup1 [52]. Finally, specific requirements of FG domain combinations for transport of various Kaps were elegantly shown in the study by Terry and Wente [22], but without any further indications of direct or indirect interactions between the specific FG domains and specific Kaps.

To map the physical routes of translocation inside yeast NPCs is difficult due to the lack of direct ways to observe the process and also a lack of structural information in yeast. We therefore rely indirectly on data gained mostly in mammalian or amphibian systems. By using a combination of fluorescently labelled cargoes in various concentrations to saturate particular transport routes, the existence of separated routes for diffusions of small molecules and active transport of large particles was proposed in mammalian cells [53]. Using atomic force microscopy, Kramer et al. [45] also found evidence for spatial separation of facilitated transport through the central channel from diffusion of ions through the pore periphery in Xenopus oocytes in accord with the original three-dimensional models [2,54]. On the other hand, Keminer and Peters [27] used an optical single transporter recording to detect a common physical pathway for both passive and facilitated transport through the central channel. The centrally located route for active translocation of at least some proteins such as colloidal gold particles coated with nucleoplasmin has been proven repeatedly by electron microscopy studies [55,56]. Our immunoelectron microscopy results with GFP (green fluorescent protein)–cargo-transformed yeast to follow the routes of various Kaps through the NPCs showed that most translocation processes occurred close to the periphery of the NPC (J. Fiserova, unpublished work).

Controversy does not avoid the export of RNA either. Studies in mammalian cells showed that RNA undergoing export entered the NPCs from the sides of the basket [57] rather than from the central axis as previously shown in Chironomus [58]. Given the variability of results achieved in different laboratories using various methods and model organisms, the
question of passive diffusion and physical translocation of Kaps–cargo through the NPC is not yet settled. A systematic interdisciplinary approach to investigate the translocation in vitro and, importantly, in vivo may be the promising way to finally understand the complex processes.

**Funding**

This work was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/E015735/1].

**References**


Received 31 July 2009
doi:10.1042/BST0380273

©The Authors Journal compilation ©2010 Biochemical Society