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Minireview

# Molecular mechanism of translocation through nuclear pore complexes during nuclear protein import

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**Abstract The trafficking of macromolecules between cytoplasm and nucleus through nuclear pore complexes is mediated by specific carrier molecules such as members of the importin-β family. Nuclear pore proteins (nucleoporins) frequently contain sequence repeats based on FG cores and carriers appear to move their cargo through the pores by hopping between successive FG cores. A major question is why some macromolecules are transported while others are not. This selectivity may be generated by the ability to bind FG repeats, a local concentration of carrier–cargo complexes near the entrance to the pore channel, and steric hindrance produced by high concentrations of nucleoporins in the channel.** 

*Key words:* Nuclear trafficking; Nucleoporin; Molecular interaction; Cell biology

## 1. Overview of nuclear trafficking

The division of eukaryotic cells into nuclear and cytoplasmic compartments raises the problem of how macromolecules are selectively transported between the two compartments. For example, nuclear proteins that are synthesised on cytoplasmic ribosomes need to be imported into the nucleus, whereas mRNA, tRNA and rRNA need to be exported from the nucleus. The trafficking of macromolecules, ions and small molecules between the cytoplasm and nucleus is mediated by nuclear pore complexes (NPCs), cylindrical proteinaceous structures, about 120 nm in diameter and 70 nm thick, that perforate the nuclear envelope (reviewed by [1–5]). Although small molecules may diffuse between the two compartments, macromolecules greater than about 40 kDa are transported actively along the central axis of the NPC. This active transport requires an appropriate signal: for example, proteins containing a nuclear localisation sequence (NLS) are imported, whereas those containing a nuclear export sequence are exported to the cytoplasm.

Although there are several different nuclear trafficking pathways, they share a number of common features (reviewed by [1–7]). Generally, substrates or cargoes do not interact directly with NPCs, but instead are transported bound to soluble carrier molecules, which are then recycled back to the original compartment. The interaction of carriers with their cargo is orchestrated by the Ras-family GTPase Ran (reviewed by [2,4,8]), although the nuclear import of Ran itself is mediated by NTF2 [9,10]. Because Ran has low intrinsic rates of nucleotide exchange and hydrolysis [11], its nucleotide state is determined primarily by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Thus, Ran GTPase activity is stimulated by cytoplasmic RanGAP1, whereas the Ran GEF, RCC1, is nuclear [2,4,8]. This spatial separation of GAP and GEF activities is thought to result in cytoplasmic Ran being primarily GDP-bound, whereas nuclear Ran is primarily GTP-bound. Although the nucleotide state of Ran regulates its interactions with other proteins, GTP hydrolysis by Ran does not appear to be linked directly to transport [12–15].

In the classic nuclear protein import pathway illustrated in Fig. 1, proteins containing a NLS first attach to soluble carriers of the importin-β/karyopherin-β family, either directly or via an adapter such as importin-α. The importin–cargo complex docks at the cytoplasmic face of the NPC, before translocating through the central channel into the nucleus. Following translocation, cargo is displaced from the carrier by nuclear RanGTP, after which the carrier, complexed with RanGTP, is recycled back through NPCs to the cytoplasm where RanGAP promotes RanGTP hydrolysis and thus dissociates the complex [1–6]. Analogous pathways have been identified for the nuclear export of RNA and protein [1,2], although mRNA export is mediated by carriers that are not importin-β homologues [16].

There is now considerable information available on the structures of many of the carriers involved in nuclear trafficking [17–21] and on the way Ran modulates interactions between carrier and cargo molecules in both the nucleus and cytoplasm (reviewed by [2,4,8]). However, the precise mechanism by which carrier–cargo complexes are translocated through NPCs remains controversial. Because it has been the most thoroughly investigated, this review will concentrate on nuclear protein import. However, the general principles governing translocation are probably similar for other nuclear trafficking pathways.

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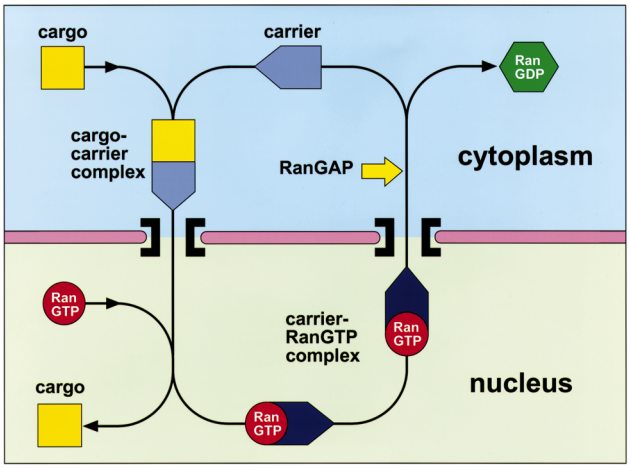


Fig. 1. Schematic model for nuclear protein import. Carrier (such as importin-β) binds to cargo in the cytoplasm, docks at the NPC and is translocated to the nucleus where RanGTP dissociates the cargo–carrier complex. The carrier–RanGTP complex is then exported to the cytoplasm where RanGAP activates the RanGTPase activity and dissociates the carrier–Ran complex, thus freeing the carrier for a further cycle of nuclear import. Reproduced with permission from Bayliss et al. [4].

## 2. Trafficking through nuclear pore complexes

NPC morphology has been reviewed extensively elsewhere [3,5]. NPCs are constructed from an approximately cylindrical central body sandwiched between nucleoplasmic and cytoplasmic rings. The central body is constructed from eight spoke-like segments and has prominent eight-fold rotational symmetry. In addition, fibres extend into both cytoplasm and nucleus and, in the case of the nuclear fibres, form a basket-like structure below the body of the NPC. The body of the NPC has a central channel through which macromolecules are transported, although the precise details of this feature are controversial. En face electron micrographs of both negatively stained and vitrified NPCs show a central cylindrical particle present in some but not others in the same field [22–24]. Three-dimensional reconstructions obtained from vitrified specimens were originally interpreted in terms of these particles representing a central 'transporter' channel [23], but more recently it has been suggested that the central material may instead represent macromolecules in transit [3]. Regardless of the precise morphology of the central channel, its limiting functional diameter appears to be 40 nm [25], somewhat larger than the putative 'transporter', but consistent with the less dense area seen in the centre of NPCs that lack a central granule [22,23].

NPCs are constructed from multiple copies of a relatively small number of proteins. For example, isolated yeast NPCs contain only 40 different proteins, called collectively nucleoporins, present mainly in either eight or multiples of eight copies [26,27]. There appears to be a level of redundancy between different nucleoporins since many *Saccharomyces cerevisiae* nucleoporin nulls are viable, albeit they frequently show synthetic lethality with other nucleoporins [28]. Vertebrate NPCs are somewhat larger than those in yeast and are probably constructed from 50–70 different proteins. Nucleoporins frequently contain FG sequence repeats, large regions consisting of tandem repeats based on highly conserved cores, containing one or two phenylalanines linked by hydrophilic spacers of variable sequence but rich in charged and polar residues [26–28]. The two most common cores are GLFG or FxFG (where x is usually serine, glycine or alanine) and some nucleoporins contain over 20 copies of these repeats. FG repeat regions of nucleoporins may not have a large amount of regular structure in solution and so may be very flexible [29,30].

Nucleoporin FG repeats appear to be directly involved in nuclear trafficking (reviewed by [4,27]). Nuclei assembled from *Xenopus* egg extract depleted of wheat germ agglutinin (WGA)-binding nucleoporins do not import NLS-containing substrates, but import is restored by adding back material eluted from WGA-Sepharose [31], indicating that either the WGA-binding FG nucleoporins, or proteins bound to them, are required for transport. Also, nuclear trafficking is blocked by antibodies that recognise FG repeats or by added nucleoporin repeat constructs [32,33], and reduced rates of nuclear protein import are seen using importin-β mutants that have reduced affinity for FxFG nucleoporins [29]. However, different transport substrates appear to be transported via different routes using particular subsets of nucleoporins [33–35] so that, although many nucleoporins are common to several trafficking pathways, others are only required for a specific pathway [27,35].

Electron microscopy has indicated that FG nucleoporins are located on both the nucleoplasmic and cytoplasmic faces of NPCs and may also line the central channel [3,26,36,37]. Pre-embedding studies using C-terminally tagged yeast nucleoporins have indicated that the majority are located symmetrically on the nuclear and cytoplasmic faces of NPCs, with only a few being restricted to one face [3,26,37]. Only Nup60p and Nup1p are exclusively nucleoplasmic, whereas only Nup159p and Nup42p are exclusively cytoplasmic [26]. Although the localisation of many nucleoporins is somewhat diffuse, some are clearly further away from the plane of the nuclear envelope, whereas others are on the nuclear basket or cytoplasmic fibrils rather than in the body of the NPC. Also, some labels are seen at higher radii than others. This diffuse localisation is consistent with the FG repeat-containing regions of nucleoporins having little regular structure and so being very flexible [29,30]. Although the use of tagged proteins gives high specificity, it only shows the location of the C-terminus. Moreover, because the colloidal gold marker has to be added before embedding, tags in some locations (especially any in the transport channel) may not be accessible. Complementary post-embedding studies using antibodies that label FxFG repeats indicate that FG nucleoporins are found in the central channel as well as on both faces of NPCs [36]. NTF2, which binds to FxFG repeats, shows a similar pattern of binding to the central channel as well as to the nuclear and cytoplasmic faces of NPCs when microinjected into *Xenopus* oocytes [25,38], or when visualised in HL60 cells using immunogold labeling [39]. Gold conjugates of NTF2 mutants that bind FxFG repeats less strongly show reduced NPC binding and are only rarely found in the central channel [38]. Overall, localisation studies indicate that FG nucleoporin repeats line both the central channel as well as the nucleoplasmic and cytoplasmic faces of NPCs.

## 3. Molecular basis of translocation in nuclear protein import

Nuclear protein import can be separated experimentally

into two steps: an initial binding to the cytoplasmic face of the NPC, which requires the presence of an NLS but not metabolic energy, followed by a separate translocation step that is reversibly arrested by metabolic inhibitors, chilling or WGA [40,41]. In the absence of ATP, cargo is located primarily at the cytoplasmic face, indicating that energy is required either for translocation or for release from the nuclear face of the NPC. Importin-β mutants deficient in RanGTP binding arrest at the nucleoplasmic face, consistent with either Ran being involved in the release of the carrier–cargo complex from the NPC, or the RanGTP-induced dissociation of the cargo–carrier complex terminating translocation [42]. Nuclear envelopes in *Xenopus* oocytes microinjected with colloidal gold coated with NLS-containing proteins show binding at a number of sites during translocation [41,43–45]. Moreover, when these nuclear envelopes are isolated and disrupted, colloidal gold particles remain attached to both the rings and the central cylinder of the NPC [46], consistent with their binding to a series of sites during translocation.

A central question is whether material is moved mechanically through NPCs, or alternatively cargo–carrier complexes simply diffuse through the central transport channel. Although nuclear trafficking of macromolecules is an active process, a number of carriers can shuttle between nucleus and cytoplasm without using energy [12–15]. In addition, measurements of transport rates in permeabilised cells indicate that translocation is extremely rapid (of the order of 1000/s) and the calculated velocity is about a quarter that expected for diffusion [47]. These observations suggest that metabolic energy may be used indirectly rather than to move material mechanically through NPCs. Thus, rather than moving carrier–cargo complexes through the central NPC channel, energy could be used to sort which material is transported [7] or to prevent return of cargo following transport. For example, if cargo–carrier complexes diffused freely in either direction through the central channel, the concentration on either side of the NPC would be the same. However, at the nucleoplasmic face, importin-β could bind RanGTP which is thought to produce a conformational change that reduces its affinity both for FxFG nucleoporins [29] and cargo [48], thus preventing diffusion of the cargo–carrier complex back to the cytoplasm. Therefore, in this very simple model, Le Chatelier's principle could drive import in a manner analogous to the way in which phosphorylation by phosphoenol pyruvate drives glucose import.

## 4. The role of FG nucleoporins in nuclear protein import

Although many studies have demonstrated interactions between carriers and FG nucleoporins in vitro and in vivo (reviewed by [27]) and have indicated that such interactions are important in nuclear trafficking, the precise way in which these interactions function remains controversial. Thus, as illustrated in Fig. 2, it has been proposed that FG nucleoporins could concentrate material to be transported near the entrance to the channel [26], or that material is translocated through the NPC by hopping between FG repeats [4,29,38,49]. Alternatively, FG nucleoporins could form a barrier to the transport of molecules [4,26,47]. These roles are not mutually exclusive and it may be that interactions between transport factors and FG nucleoporins serve several different roles.

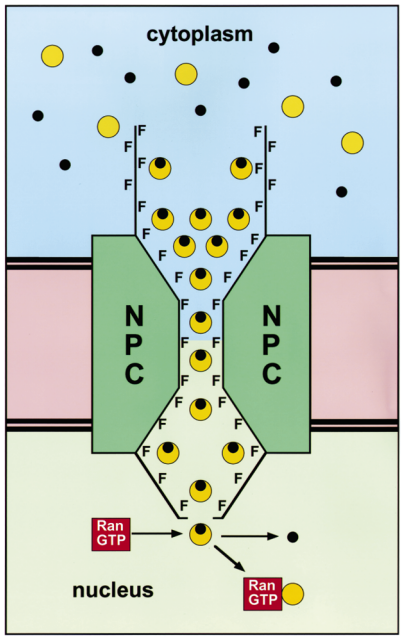


Fig. 2. Schematic illustration of how interactions between FxFG nucleoporins and cargo–carrier complexes could facilitate nuclear protein import. Nuclear pore complexes have a central channel with cytoplasmic and nucleoplasmic vestibules to which fibres are attached. FxFG nucleoporins, represented by F, are found in the cytoplasmic and nucleoplasmic fibres as well as lining the vestibules and central transport channel of the NPC. Carrier molecules, represented as yellow circles, bind cargo (smaller black circles) in the cytoplasm and are concentrated by the fibres at the cytoplasmic entrance to the transport channel. Cargo–carrier complexes are translocated through NPCs by hopping between FxFG nucleoporin cores and are then removed from the nucleoplasmic face by binding RanGTP, which prevents binding to the FxFG nucleoporin cores and dissociates the cargo–carrier complex. Carrier bound to RanGTP is then recycled to the cytoplasm (see Fig. 1).

The hypothesis that cargo–carrier complexes hop between FG nucleoporin repeats lining the central channel is supported by the observation that material is attached at a number of points through NPCs during transport [41,43,44]. Facilitation of transport by attachment of substrate at a number of sites along a channel is also seen in a range of other transport systems. Crystal structures of the glycerol [50] and potassium [51] channels, for example, show the crucial importance of interacting with channel components and of having multiple weak binding sites along the channel to which the substrate binds sequentially. Analogous interactions are also seen with steroid hormones that pass through membranes because they have similar solubilities in water and lipid. Thus there is not a substantial energy barrier for their moving from one phase to another. Similarly, the passage of macromolecules through NPCs is unlikely to involve a major energy transition because the environment in the channel is probably very similar to that in cytoplasm, with high protein concentration (the concentration of FxFG cores is probably of the order of 50 mM in the channel [38]) and generally hydrophilic environment produced by the FG nucleoporin repeat linkers. Although water in the transport channel is likely to be structured, it is probably not greatly different to the cytoplasm [52]. Accordingly, weak interactions with FG nucleoporins could facilitate movement of carriers and their cargo through NPCs (Fig. 2). It is important that interactions are weak (with correspondingly high off-rates) to enable high rates of transport

(see [38,53,54]). Translocation may be by simply hopping between FG cores or may be biased by an affinity gradient [54].

The hypothesis that the FG nucleoporins function to concentrate cargo–carrier complexes near the entrance to the transport channel is supported both by the high concentration of FG nucleoporins found on the cytoplasmic and nucleoplasmic faces of NPCs [26], and by the observation that the initial binding of import cargo–carrier complexes takes place at the cytoplasmic face, most notably at the cytoplasmic fibres [41]. Analogous concentrating mechanisms are seen in other channels where, for example, the aquaporin and potassium channels often have a vestibule at their entrance that may function either to direct substrate to the transport channel or to effect a local concentration of the substrate at the channel entrance [50,51]. Concentrating material at the NPC entrance would substantially increase its flux through the central channel. The fibres that extend from each face of NPCs could be particularly important in such a concentrating effect, since fibrils adsorb material at a surprisingly high rate [55] and one-dimensional diffusion along the fibres to the entrance of the NPC would be rapid. Two vertebrate FxFG nucleoporins to which importin-β binds (CAN/Nup214 and Nup356) are located in cytoplasmic fibrils [3]. In addition to the fibres, NPCs have large entrance vestibules on both faces that may function like antennae to concentrate particles near the mouth of the pore. In addition to these morphological factors, the location of RanGAP on fibrils near the cytoplasmic entrance vestibule would generate a local high concentration of Ran-free importin-β able to bind cargo. Because of the dimensions of both NPCs and substrates together with the effects produced by molecular crowding [52], such a concentrating effect may be more important for nuclear trafficking than in some other transport systems.

Nuclear protein import is inhibited by antibodies to FG nucleoporins, addition of endogenous nucleoporins [29,33,34], and by mutants of NTF2 and importin-β that bind less tightly to FxFG nucleoporins [29,38,47]. However, these results do not define the precise stage of the transport mechanism at which the interaction between transport factors and nucleoporins is important (and indeed these interactions could be important at more than one step). One view is that the FG nucleoporins are located only on faces of NPCs and so involved in either local concentration of carriers and/or steric obstruction of material to be excluded [26]. In this model, translocation through the NPC is proposed to be simple diffusion through an aqueous channel. However, FG nucleoporins are probably also located in the central transport channel [36,38], and the observation that nucleoplasmin-coated colloidal gold remains in the central channel of isolated NPCs from which the nucleoplasmic and cytoplasmic rings have been removed [46] is more consistent with their binding directly to the channel. Although colloidal gold coated with W7A-NTF2 (which shows reduced FxFG nucleoporin binding and nuclear trafficking) still concentrates at the nucleoplasmic and cytoplasmic faces of NPCs, it is excluded from the channel [38], indicating that it is unlikely that the nucleoporin–carrier interaction is involved only in concentrating material prior to translocation. Conversely, the observation that nuclear protein import can be inhibited by added FG nucleoporins [29,33,34] is difficult to reconcile with the nucleoporin–carrier interaction being involved only in translocation and instead support its having some role in the initial binding of the complex to the NPC. Overall therefore it seems probable that interactions between FG nucleoporin repeats and carriers will function in both the initial binding to the NPC and translocation through it (Fig. 2), albeit different nucleoporins could be involved in each step.

## 5. Molecular basis of transport selectivity

It is paradoxical that NPCs are able to exclude molecules of the order of 40–50 kDa (or about 5 nm diameter) when the limiting diameter of the central transport channel is of the order of 40 nm [25]. It is not clear precisely how stringent selectivity at the NPC is in nuclear trafficking. Although there is a substantial difference in the net rate of transport of appropriate and inappropriate macromolecules, additional selectivity could be provided by, for example, export of inappropriately imported material or retention by binding to structures in one compartment or the other. One possible mechanism for generating transport selectivity is that the channel is gated, so that it is normally closed and only opens for substrate. Gating is commonly used to impose selectivity in the transport of small molecules through channels. For example, in both glycerol and potassium channels, there is a narrow constriction where only the correct substrate is able to form suitable interactions with the channel protein to compensate for loss of hydration [50,51]. Because of the great variety of macromolecules transported, it seems unlikely that such a recognition mechanism would be used for nuclear trafficking. In principle, the NPC channel could open and close to allow only appropriate cargo–carrier complexes to pass, but there is little structural evidence for gross conformational changes in NPCs consistent with their opening only for transport. Moreover, it is difficult to reconcile such a gating mechanism with the observations that several cargo–carrier complexes can be transported at the same time [41,43] and that import and export can occur simultaneously in the same pore [44].

It may be that selectivity of transport can be accounted for by the same mechanisms used to facilitate passage of cargo–carrier complexes through NPCs. Thus, only appropriate cargo–carrier complexes would concentrate at the entrance and be translocated through NPCs by interacting with FG nucleoporins. These two factors acting in concert would certainly increase the flux of carrier–cargo complexes substantially compared with macromolecules that did not bind to FG nucleoporins. Because they would not bind to FG nucleoporins, inappropriate molecules would not concentrate at the entrance to the NPC transport channel and so their rate of transport would be much lower than cargoes bound to carriers. In addition, the ability to interact with FG repeats in the transport channel could help facilitate transport and increase the flux of cargo–carrier complexes. But would such a mechanism be sufficient to explain the observed selectivity or would there also need to be some sort of mechanism whereby other molecules are excluded from transport? In this context, it has been proposed that the FG nucleoporins may obstruct the passage of molecules that do not interact with them. One way in which nucleoporins could obstruct is by their sheer bulk, so that they would generate a form of 'entropic exclusion' [26]. However, in electron micrographs of NPCs lacking a central granule, the central channel seems to have approximately the same density as the cytoplasm and nucleoplasm

(albeit the rest of the pore is more dense). Although the concentration of FxFG repeats in the channel has been estimated to be of the order of 50 mM [38], this would only correspond to a protein concentration of about 100–200 mg/ml. The cytoplasm has a protein concentration of the order of 200–300 mg/ml and is therefore already extremely crowded [52], and so it is not clear how the FG nucleoporins alone could offer more obstruction than that already present in the cytoplasm. One possibility would be the suggestion that the FG cores of nucleoporin repeats could interact with one another to generate a tightly crosslinked gel [47]. This gel could act as a selective phase which allowed the passage of proteins that could bind to the cores (and so break the crosslinks), but prevented the passage of other macromolecules. Although this is an attractive concept, there does not appear to be direct biochemical or structural evidence for an interaction between FG repeats that could generate such a crosslinked gel phase in the transport channel.

## 6. Future directions

Nuclear trafficking can now be generally understood in terms of facilitated diffusion of cargo–carrier complexes through NPCs in which metabolic energy is used indirectly to specify directionality and selectivity. Although many aspects of the molecular mechanism of nuclear trafficking have been established, the precise mechanisms by which selectivity is achieved and by which material is translocated through NPCs remain to be resolved. Future work will need to establish the relative importance of steric obstruction, local concentration at NPC faces and translocation in establishing selectivity. It will also be crucial to obtain structural information on a range of FG nucleoporins to see how flexible the repeat regions are, as well as establishing the precise location and density of FG repeats within NPCs. In addition, it will be necessary to establish which carriers interact with which nucleoporins and whether there are gradients of interaction that may have a role in establishing the directionality of transport.

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