ELSEVIER

Virtual gating and nuclear transport: the hole picture

Michael P. Rout[1], John D. Aitchison[2], Marcelo O. Magnasco[1] and Brian T. Chait[1]

[1]The Rockefeller University, New York, NY 10021, USA
[2]Institute for Systems Biology, Seattle, WA 98105, USA

The eukaryotic nucleus is surrounded by a protective nuclear envelope, which is perforated by trafficking machines termed nuclear pore complexes (NPCs). The NPCs are the sole mediators of exchange between the nucleus and the cytoplasm. Small molecules pass through the NPCs unchallenged; however, large macromolecules are excluded unless chaperoned across by transport factors. Here, we suggest a model, termed 'virtual gating', to explain the mechanism of this rapid and selective macromolecular trafficking.

In eukaryotes, the double-membraned nuclear envelope (NE) separates the nucleoplasmic and cytoplasmic compartments, sequestering the genetic material in the nucleus. This provides the cell with the opportunity to control access to its DNA, a defining feature of eukaryotes. Although the NE membranes act as the barrier, it is the nuclear-pore complexes (NPCs) embedded in the NE that serve as highly versatile gates (Figure 1). NPCs provide little obstruction to the free exchange of small molecules, such as water and ions, between these compartments; but, the larger a molecule is, the more its movement across the NPC is restricted, such that the majority of proteins with molecular weights above ~40 kDa are essentially prevented from crossing the NE. On the other hand, NPCs can direct the accumulation of selected macromolecules in either compartment, often against a significant concentration gradient [1–5]. How is this gating achieved? Here, we describe a model, termed 'virtual gating', to account for transport through the NPCs [6].

Putting the model in context

The signal hypothesis and the karyopherin cycle

Generally, proteins larger than ~40 kDa must be specifically transported through the NPCs. This transport is receptor-mediated (and is therefore selective), energy dependent and fast [7–9]. Transport involves the recognition of nuclear localization signals (NLSs) on cargos destined for the nucleus and nuclear export signals (NESs) on cargos destined for the cytoplasm. Most import and export signals are recognized by the β-karyopherin (kap) family of soluble transport receptor proteins (also known as importins and exportins). Once bound to a cargo, a kap negotiates the NPC, releases its cargo in the destination compartment and returns for another round. Because this cycle is directional and can accumulate cargos against a concentration gradient, an energy source and a directional cue are needed. Both are provided by the small GTPase Ran, which is controlled by two regulators – Ran guanosine nucleotide exchange factor (RanGEF), and its 'alter ego' Ran-specific GTPase activating protein (RanGAP) [2,5,10–17] (Figure 1). But, how does the NPC select only the karyopherin–cargo complexes and exclude other macromolecules? In other words, how does the NPC act as a sorting machine?

Macroscopic and microscopic machines

Consider molecular machines within a cell. On the macroscopic scale, machines are dominated by properties such as friction, heat exchange, leverage and flow. However, on the microscopic scale the emphasis changes, and molecular machines are dominated by properties such as diffusion and viscosity [18]. Thus, in considering how one might build a microscopic machine, analogies with macroscopic machines might not be helpful. For example, imagine a protein in a yeast cell. If this cell was scaled up (with all its microscopic properties) to the size of a lake, a single protein molecule would be roughly the size of a person, surrounded by water molecules the size of baseballs. While a person may swim sedately across a lake, diffusion will make our protein rocket randomly around the lake at many times the speed of sound [19]. Getting macromolecules around in a small cell, therefore, is not a major issue. Instead, the problems lie in keeping selected macromolecules at their destination once they arrive and excluding undesirables; indeed, it appears that these requirements dictate the design of the NPCs [20].

The blueprint of the machine

To early electron microscopists, NPCs appeared as apertures in the NE formed where the two NE membranes fuse. As techniques improved, a more detailed picture emerged of a highly conserved cylindrical assembly ~50 MDa in mass and ~100 nm in diameter. Higher-resolution images revealed a central tube with a diameter of ~30 nm connecting the nucleoplasm and the cytoplasm, through which the exchange of macromolecules occurs. The tube is surrounded by eight radial spokes, composed of elaborate struts and buttresses and conjoined coaxially by rings [21,22]. A considerable portion of each spoke is seen to penetrate the aperture membrane and extend into the lumen of the NE. Together, these

Corresponding author: Michael P. Rout (rout@rockefeller.edu).

 0962-8924/$ - see front matter doi:10.1016/j.tcb.2003.10.007

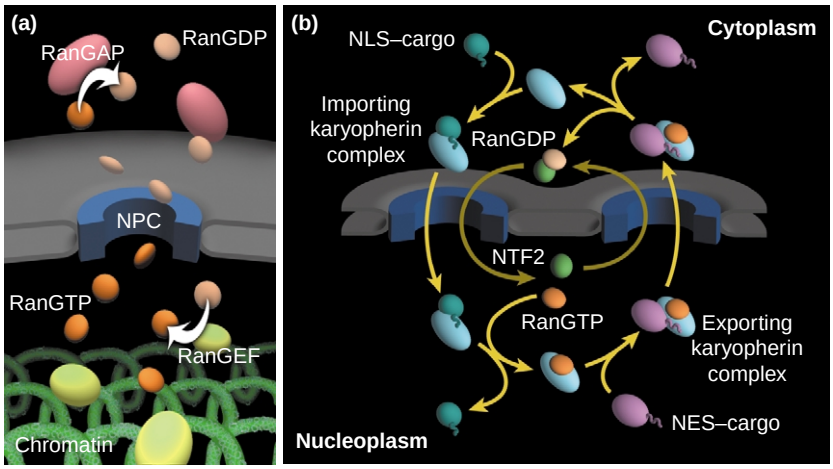

Figure 1. The mobile phase of nuclear transport. **(a)** Setting up the RanGTP–GDP gradient across the nuclear envelope (NE). RanGEF loads Ran with GTP, whereas RanGAP encourages Ran to hydrolyze GTP. RanGEF strongly binds to chromatin and so flags the position of chromatin in the cell. By contrast, RanGAP is found largely in the cytoplasm. The result is that in the vicinity of chromatin (i.e. in the nucleoplasm) one finds mostly Ran bound to GTP whereas cytoplasmic Ran is mainly found in its GDP-bound form. This gradient powers much transport across the nuclear pore complex (NPC). **(b)** The nuclear transport cycle. An importing karyopherin (kap) binds to its NLS-bearing cargo in the cytoplasm and transits the NPC. On the nucleoplasmic side, RanGTP binds to the kap, causing a conformational change that releases the cargo. In the nucleoplasm, exporting kaps bind their cargos in the presence of RanGTP. Once the exporting complexes are on the cytoplasmic side, RanGTP hydrolysis is stimulated by RanGAP, resulting in the release of cargo. RanGDP is then recycled to the nucleoplasm by NTF2 and is reloaded with GTP to begin another cycle.

structures comprise the cylindrical core. Numerous extensions bristle from this core, projecting into the nucleoplasm and cytoplasm [1,4,23].

It was originally thought that the complicated structure of NPCs required hundreds of different components to construct a mechano–chemically gated portal and to support an elaborate series of energy-driven binding and exchange reactions However, recent compositional and architectural surveys have forced a rethink (Figure 2). NPCs from both yeast and vertebrates are compositionally similar and surprisingly simple, each being made of ~30 distinct components [6,24,25]. Most of these component proteins, termed nucleoporins or nups, are present in two copies per spoke (with eight spokes per NPC), each copy

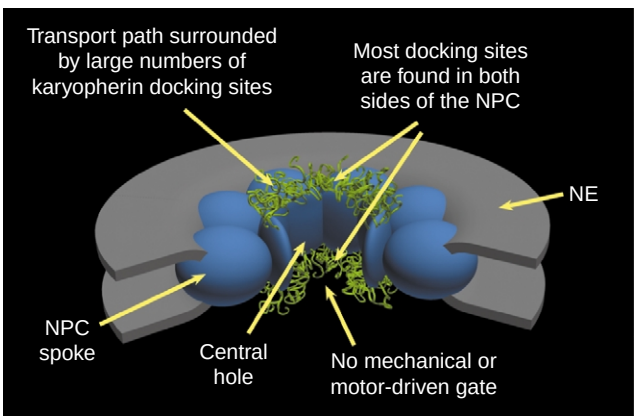

Figure 2. Diagrammatic representation of the nuclear pore complex (NPC). Recent studies indicate a surprisingly simple architecture for the NPC. It lacks proteins normally associated with mechano–chemical transport; instead a large number of closely packed binding-site proteins surround the transport path, most of which are found on both the nuclear and cytoplasmic sides of the NPC. This diagram is highly simplified, retaining only the features we believe to be central to the virtual gating model. Other structures (e.g. baskets, cytoplasmic filaments, central transporters [1]) are omitted for the sake of clarity.

located symmetrically on either side of the NPC midplane. This way, the NPC can attain a large size with a relatively small number of components. Perhaps the biggest surprise came from finding no ATPases or GTPases among the list of nups. Indeed, several lines of evidence indicate that, while the Ran cycle provides the main source of energy to sustain directional transport, no nucleotide hydrolysis or mechano–enzyme activity is needed to gate translocation itself [26–29].

In the absence of mechano–enzymes, such as myosin, there are only three classes of nups with which we can explain transport. The first class is a set of membrane proteins, called poms, which anchor the NPC into the NE. The members of the second class are most probably structural proteins, giving NPCs shape and strength. These proteins form the central tube and provide a scaffold for the deployment of the third class of nups across both faces of the NPC. This third class provides binding sites for transport factors. They are a related group of proteins, collectively termed FG nups because they contain multiple copies of a Phe-Gly motif separated by hydrophilic residues [3,4]. Although most FG nups are distributed symmetrically on the nuclear and cytoplasmic faces of the NPC, a few are preferentially found on one face or the other. Nearly half the mass of NPCs can be accounted for by FG nups [6]. Curiously, FG-containing regions appear to have a string-like disordered structure and seem to be the major constituent of the bristling extensions covering the two faces of the NPC [23,30,31]. Amazingly, ~200 copies of FG nups are found in each NPC [6,24,25], providing the main binding sites for transport factors. Thus, despite their elaborate architecture, the membrane and core structures of NPCs can be considered a framework that ensures the correct positioning of the binding sites that directly mediate transport. We picture the NPC as a tubular hole in the NE, bristling at each entrance with numerous filaments carrying a multitude of binding

sites for transport factors. But, can such a simple structure mediate all the complexities of gated transport? Indeed, it can.

Virtual gating

A hole can be a barrier, even if a molecule is small enough to pass through it

Consider the entropy of a macromolecule, where entropy can be thought of as the number of ways to distribute the energetic motions of the macromolecule. Consider also a macromolecule freely diffusing within the cytosol. A molecule has many possible places to go and several ways to move around, hence its entropy is high. However, in the confined volume encompassed by the central tube of the NPC its movement is highly restricted and therefore its entropy has decreased. Thus, an entropic price must be paid to place a macromolecule within the central tube. As the size of a macromolecule increases, the entropic price it has to pay to pass through the central tube rises, and the probability of its passage through the NE decreases. Above a certain size this probability becomes negligible, and the NPC is effectively impermeable (Figure 3a). The densely packed FG nups probably add to this entropic price, by further constraining the free space available for diffusion at the NPC. Although the string-like structure of FG nups might permit them to move aside and allow macromolecules to pass, this would require some energy. It is easy to imagine how this crowded region of FG nups could be made impassable for a passively diffusing object above a limiting diameter. Other factors might also contribute to the building of this permeability barrier (Box 1).

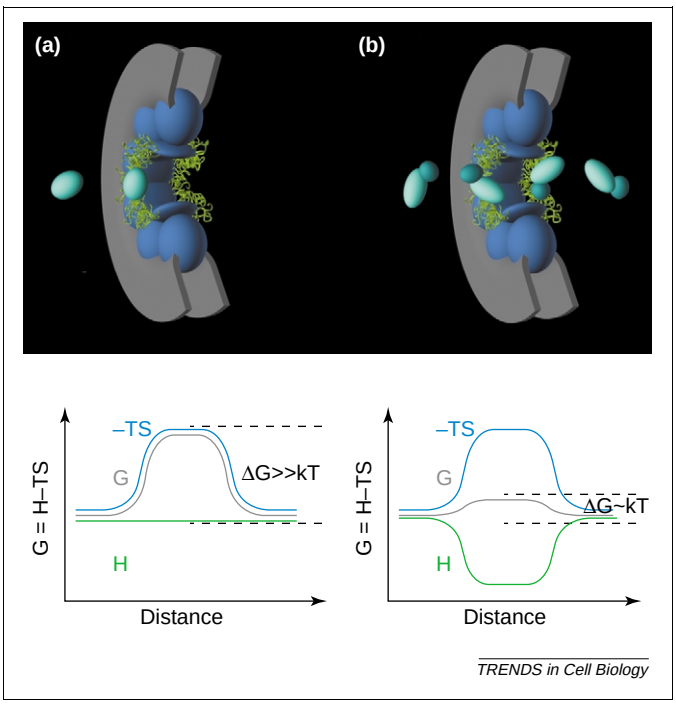

Figure 3. Energetics of macromolecular diffusion across the nuclear pore complex (NPC). Illustrations show **(a)** a macromolecule (turquoise) incapable of binding the NPC (blue), and **(b)** a similarly sized karyopherin–cargo complex (light and dark turquoise) able to bind FG nups (green) on the NPC. Graphs (bottom) showing the energetics of the same processes (see text for an explanation).

Box 1. The fine details of virtual gating

We do not know the fine details of how the entropic barrier is set up. Beyond simple occlusion, other factors could add to the barrier properties of the NPC. The intrinsically disordered FG nups could act as 'entropic bristles' [41] – diffusive forces could cause them to whip and writhe around their anchor points at the NPC, allowing them to explore a large volume around their tether site and in essence 'fill up' this volume. Molecules that are large enough to occupy a significant portion of this volume and move on the same timescale as the bristles tend to be excluded from this volume. The disordered filamentous sidearms of neurofilaments and microtubule-associated proteins act as entropic bristles, whose 'push' might help to keep the parallel arrays of their associated filaments regularly spaced [42,43]. Similarly, the 'push' from the FG nups could keep macromolecules away from the central channel, and the larger the macromolecule, the more it would feel this push [6]. The appeal of this model is that it uses a well-studied polymer phenomenon consistent with the reported structure of FG nups. Several alternative proposals have been made, including the oily-spaghetti model [2], the selective-phase model [8,36,44] and the molecular-latch model [45]. Aspects of all these ideas might contribute to virtual gating, but more experimentation is needed.

The barrier can be lowered by binding

In theory, any macromolecule smaller than 30 nm could part the FG nup curtain and pass through the narrow channel it protects as long as the macromolecule can afford to pay the entropic penalty for doing so. As a macromolecule must pass through this region to get from one side to the other, being within the NPC can therefore be considered a sort of 'transition state' for translocation. To cross, macromolecules need to be encouraged to enter the 'transition state'. One way of doing this would be to have an affinity for and bind to this region of the NPC. Such specifically binding macromolecules thus have access to the 'transition state', (i.e. they can cross the NPC) (Figure 3b). This is exactly what transport factors do – they bind to the NPC, which allows them to access the 'transition state' of transport, and thus pass through the NPC. Conversely, macromolecules that do not bind to the NPC have an extremely low probability of accessing the 'transition state' and so effectively do not cross the NE (Figure 3a).

Consider translocation across the NPC in terms of the Gibb's free energy (G) of a system, defined as the difference between the enthalpy of the system [(H), a measure of the available energy in the system] and the product of its temperature and entropy ($T.S$) [32]. In a much-simplified consideration of our NPC system, the change in $T.S$ as a function of distance across the NE describes the entropic barrier of the NPC. The change in H describes the binding energy of macromolecules to the NPC (Figure 3). If the change in G is positive, a reaction will not proceed. In an isolated system, a process that involves a decrease in entropy and without any change in enthalpy will have a positive ΔG, and thus will not spontaneously occur. Going through a physical restriction such as a pore means temporarily losing some entropy. The positive ΔG that this entails represents an energy barrier to activation and means this process will happen at a low spontaneous rate (e.g. a nonbinding macromolecule attempting to cross the NPC). For translocation to occur, ΔG must be lowered

below the diffusion energy available to a macromolecule ($\sim k.T$). It is possible to lower this ΔG by using binding energy (ΔH) as a compensation, flattening the energy landscape (ΔG); this leads to a lower activation energy of translocation across the NE (Figure 3b) (binding also has an entropic term; nonetheless, the sum of entropies of binding and diffusion can be cancelled out by a sufficient ΔH). Too much binding energy would be counterproductive, since then the molecule would face a positive ΔG to *get out* of the pore. In an optimal situation, the energies of binding and barrier are balanced, such that a macromolecule neither accumulates at nor is excluded from the NPC but passes rapidly with minimal hindrance (Figure 3b).

The kinetics of binding

The use of binding sites to overcome an entropic barrier has its price. A binding macromolecule must spend some time attached to its binding sites, slowing its overall translocation rate across the NPC. If it takes too long, the overall process of translocation will be too slow. So what kind of binding sites must be used? Because high-affinity binding sites generally exhibit low off-rates, even a small number of such sites could be a problem at the central tube [although they may be useful elsewhere in the NPC (Box 2)]. They would retain their bound macromolecules for too long, retard their passage or even trap them at the NPC. To be effective, the binding sites surrounding the central tube must have low enough affinities, and thus high enough off-rates to allow sufficiently rapid passage of transport factors across the channel. In fact, by having a large number of low-affinity binding sites the central channel can provide sufficient binding energy to effectively lower the entropic barrier without compromising the speed of transport [2].

Bouncers at the door – the FG nups might both push away undesirable molecules and let through the paying customers

It appears that the NPC employs an elegant economy of function – FG nups, which seem to help form the barrier, are also the binding sites for transport factors. Interestingly, the predicted multitude of low-affinity binding sites for transport factors corresponds to what is observed, as the ~200 FG nups at the NPC are themselves made of multiple repeats of FG-binding sites, each with a relatively low affinity and a high exchange rate [2,8,33–35]. This provides hundreds of potential stepping stones across which transport factors can pass. The multiplicity of binding sites could also provide the NPC with the necessary capacity to bind to many transport factors simultaneously, allowing high transport flux.

Given the close proximity of so many FG repeats at the NPC, transport factors might interact with several FG repeats simultaneously. The potential even exists for a transport factor to travel 'hand-over-fist' between repeats across the NPC, always holding on to at least one FG repeat. Thus, it is not so much affinities, but avidities – the functional affinity resulting from the interaction between two molecules through multiple binding sites – that we might have to consider when attempting to derive a molecular kinetic description of the NPC [2].

The NPC as a translocation catalyst

We are familiar with enzymes acting as catalysts that function by lowering the activation energy of a reaction. Enzymes create transition states that have lowered energy, accelerating the rate of transition between substrate and product (Figure 4a). While certainly not a Michaelis–Menten enzyme, in one sense, the NPC can be likened to a catalyst, facilitating the exchange of transport–factor-cargo complexes across the NE [8,36]. As with an enzyme, this facilitation works by lowering the activation energy barrier. In the NPC the barrier is entropic and is overcome by the binding energy of specific transport factors, but, as with catalysis, this binding should be neither too weak nor too strong [37]. Like an enzyme, the lowering of the energy barrier does not favor

Box 2. Trapping – how do cargos stay put?

There are two modes by which a cargo is retained, or 'trapped', in either the nucleoplasmic or the cytoplasmic compartment. First, trapping can occur if active transport in one direction is faster than passive diffusion in the other. This is consistent with the results from experiments using nuclear localization signal (NLS)-tagged GFP, which efficiently accumulate in the nucleus, even though the GFP does not bind there and (being small enough) can passively diffuse out [45,46]. In this case, it is believed that the binding of RanGTP to the karyopherin, after it arrives in the nucleus, releases the NLS–GFP cargo. When it is free of the karyopherin, the NLS–GFP cargo can no longer bind to the nuclear pore complex (NPC) and therefore would be subject to the full effect of the postulated entropic barrier. The cargo would therefore have been converted from a rapidly diffusing form (facilitated by the bound karyopherin) to a slow-diffusing form (the NLS–GFP alone) and is thus essentially trapped.

The second mode of 'trapping' involves sequestration of a macromolecule to binding sites on one side of the NE that prevent it from diffusing back – this is the classic 'source and sink' scenario (Box 3); for example, ribosomal proteins bind nucleolar rRNAs after their import into the nucleus. It seems likely that once imported into the nucleus, if it were not for their retention by rRNAs, their small size ($\ll$ 40 kDa) would permit them to diffuse back through the NPC [2].

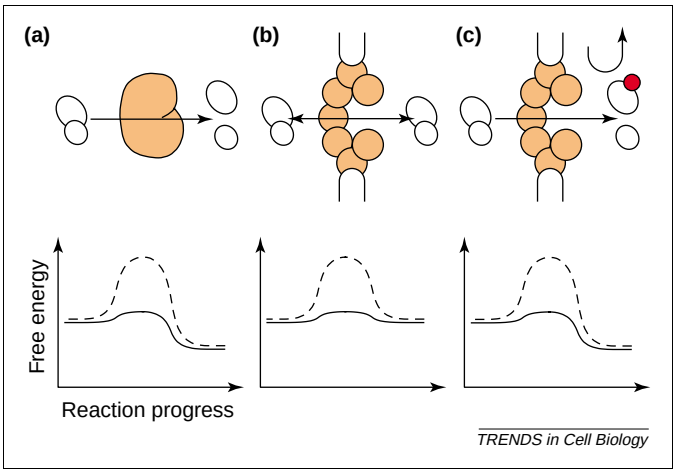

Figure 4. The nuclear pore complex (NPC) is analogous to an enzyme. **(a)** Top: a classical Michaelis-Menten enzyme (orange) converting a substrate (left) to products (right). Bottom: graph showing the Gibb's free energy of reactants and products, and the activation energy, with (unbroken line) and without (broken line) the enzyme. **(b)** The NPC (orange) catalyzing diffusion across the nuclear envelope, showing the activation energy with (unbroken line) and without (broken line) binding to the NPC. **(c)** After translocation, cargo is released from the transporter by binding of RanGTP (red). As in (a), the 'products' are at a lower free energy than the 'reactants'.

transport in any particular direction and, in the absence of any other cues, the only effect is to allow transport factors to diffuse back and forth across the NE much faster than similarly sized nonbinding macromolecules (Figure 4b).

Concentration gradients drive transport

Before their import, NLS–cargo–kap complexes constantly form in the cytoplasm. When transported into the nucleus, the high concentration of RanGTP then favors the release of cargos. This is an exothermic reaction that results in a lower final energy state (Figure 4c). The resulting concentration gradient of cargo–kap drives the complexes into the nucleus. Cargos accumulate in the nucleus because they become 'trapped' there (Box 2) [2,7,38]. In addition, the nuclear kap–RanGTP complexes, which are produced during import, form a concentration gradient decreasing towards the cytoplasm; these complexes probably diffuse into the cytoplasm (through NPC binding), where RanGAP facilitates the hydrolysis of RanGTP to RanGDP and the dissociation of Ran from kap (Figure 1). Similarly, export kaps form complexes with RanGTP and their NES–cargos in the nucleus and diffuse down a concentration gradient into the cytoplasm where RanGAP dissociates the complexes and releases their cargos. This increases the cytoplasmic concentrations of RanGDP and free kaps. It seems that free kaps, still

Box 3. Themes and variations

Evidence already exists for numerous additional or alternative mechanisms associated with nucleocytoplasmic transport to augment virtual gating and ensure efficient cargo trapping.

Different trails through the channel canyon. One reason for the large number of different FG nups might be to provide alternative pathways across the nuclear pore complex (NPC) for different transport factors. Thus, although they all go though the same tube, their use of separate binding sites decreases congestion at the NPC (Figure Ia; green kap preferring green FG nup binding sites). Furthermore, this arrangement could provide opportunities for differential regulation [23,47,48].

The pore itself is biased! Not all the binding sites on the NPC are distributed equally between the nuclear and cytoplasmic sides. Some FG nups are found preferentially or exclusively on one or the other face of the NPC. It has been suggested that these asymmetric binding sites might be used as guideposts for the directionality of movement of the transport-factor–cargo complex across the NPC. They could do this by providing a preferred high-affinity binding platform at the far end of the route of a transport factor through the NPC. Thus, once a transport factor has negotiated the central tube, it could take an essentially irreversible jump to the high-affinity FG nups, found only on the opposite face from which the transport factor started, hindering it from wandering back the wrong way through the tube again. Once held at this site, exposure to the alternative Ran milieu from which the transport factor started terminates the transport reaction. For example, in Figure Ib, an import karyopherin (light blue) is 'pulled over' to the nucleoplasm by an asymmetric FG nup on the nuclear side of the NPC (dark blue filament), where the import reaction is terminated by RanGTP (orange) [23,40,47,35,49].

Is transport always run by Ran? Some proteins appear to traverse the NPC based solely on their affinity for FG nups and for a binding site restricted to one side of the NE – the classic 'source and sink' scenario. β-catenin might be one such example, entering the nucleus by facilitated diffusion and then being retained there by binding chromatin (Figure Ic) [2,5].

Dilation of the central channel. It appears that the nuclear basket and central channel dilate in response to the translocation of large cargos (Figure Id) [21,50]. Other conformational changes in the NPC might also accompany transport [45]. Again, this is compatible with a virtual gating mechanism. The dilation might be an elastic response to the large size of certain cargos. The energy causing this dilation probably derives from the binding of transport-factor–cargo complexes to the NPC.

Getting the big stuff across. It might not be immediately obvious how the export of messenger ribonucleoproteins (mRNPs) can be accomplished by virtual gating. Among the most dramatic examples of mRNA export are the huge Balbiani-ring–mRNP particles. As it exits the nucleus, the Balbiani–mRNP complex unfurls, and the massive complex spools through from one side of the NPC to the other, similar to film through a movie camera [51,52,53]. Yet, these observed changes can easily be reconciled with a virtual gating mechanism. One can consider an RNP as being made of a string (the mRNA) threaded through a line of beads (groups of mRNA-binding proteins) (Figure Ie, pink). When transport factors bind each macromolecular bead (Figure Ie, yellow), they carry it across the NPC just the same as any other

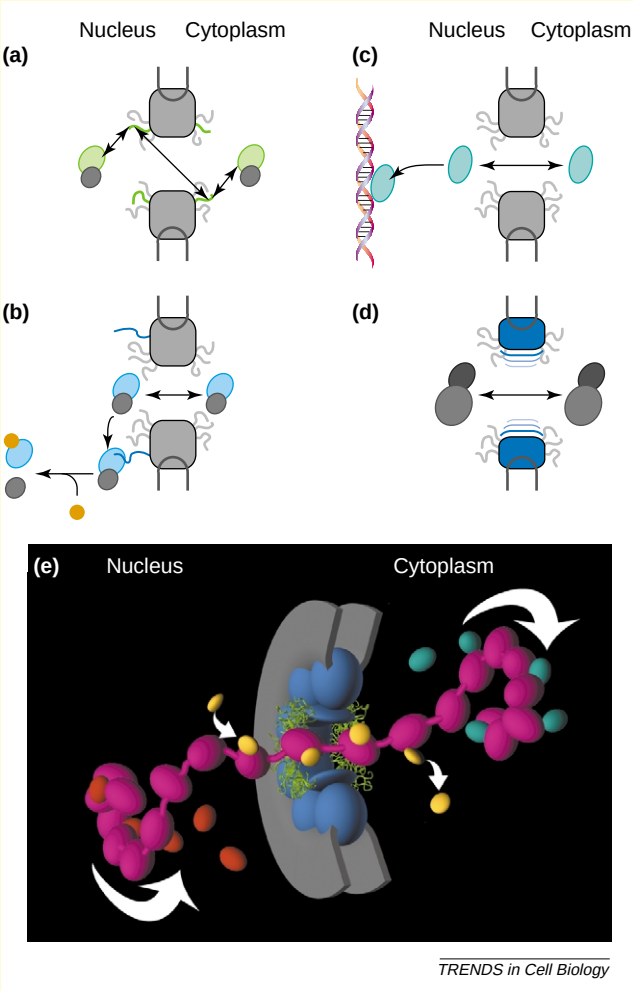

Figure I.

macromolecular assemblage, with the transport factors overcoming the entropic barrier to move the assemblage though the central channel. When the first bead is across, the next bead in line can be picked up by transport factors, and in this way the whole string of beads can be taken across. Repeated rounds of RanGTP hydrolysis might be needed [54]. ATP hydrolysis by RNA helicases could also power the process by unrolling the mRNP on the nuclear side (Figure Ie, red) and rolling it through on the cytoplasmic side (Figure Ie, green), aided by the energy released upon association of cytoplasmic proteins with the RNA.

competent for NPC binding, can rapidly diffuse across the NPC [39]. This would allow them to pass between the nucleus and cytoplasm and scour each compartment for new cargos [2]. Thus, the hydrolysis of GTP maintains the diffusion gradients that ultimately force cargos to concentrate on one or other side of the NE.

Were nucleocytoplasmic transport this simple – just a matter of entropic barriers, NPC binding and GTP-renewed concentration gradients – then it should be possible (in accordance with Le Chatelier's principle) to reverse the normal direction of transport through the NPC. Indeed, in agreement with these simple tenets, such reversal has been demonstrated [40]. Nevertheless, we must emphasize that our virtual-gating model is clearly simplified. It seems possible that different transport pathways might use different directional cues, energy sources and even additional mechanisms from those described above (Box 3). However, we believe they are all consistent with the ideas outlined here.

Concluding remarks

Numerous threads of evidence from many workers have begun to produce a coherent picture of the nucleoplasmic transport mechanism. Our virtual gating model is a consequence of this coalescence of information and is able to explain the observed major features of nucleo-cytoplasmic transport. However, a great deal of detailed work is now needed to test this and other ideas and to sort out the intricacies of this fascinating process that is so central to the lifestyles of every eukaryote.

It is probable that the principles of virtual gating are used elsewhere in the cell. Certainly, one can argue that the concepts overlap with those describing how protein-conducting channels and ion channels work, and there are many other cellular barricades that are perhaps crossed by using such a mechanism. Another aspect of future interest is that, when we understand the intricacies of nuclear transport, we can perhaps design protein- and drug-sorting machines to mimic the splendid efficacy of their molecular selection.

Acknowledgements

We thank Chris Akey, Remy Chait, Michael Elbaum, David Gadsby, Tom Muir, Kellene Mullin, Ben Timney and the reviewers for critical reading of the manuscript and many helpful suggestions. Articles have been cited where possible; however, we apologize to those many authors whose original work we could not cite because of space limitations. We encourage readers to use the reviews we have cited to lead them to this work.

References

1 Wente, S.R. (2000) Gatekeepers of the nucleus. *Science* 288, 1374–1377
2 Macara, I.G. (2001) Transport into and out of the nucleus. *Microbiol. Mol. Biol. Rev.* 65, 570–594
3 Suntharalingam, M. and Wente, S.R. (2003) Peering through the pore. Nuclear pore complex structure, assembly, and function. *Dev. Cell* 4, 775–789
4 Rout, M.P. and Aitchison, J.D. (2001) The nuclear pore complex as a ransport machine. *J. Biol. Chem.* 276, 16593–16596
5 Weis, K. (2002) Nucleocytoplasmic transport: cargo trafficking across the border. *Curr. Opin. Cell Biol.* 14, 328–335
6 Rout, M.P. *et al.* (2000) The yeast nuclear pore complex: composition, architecture, and transport mechanism. *J. Cell Biol.* 148, 635–651
7 Smith, A.E. *et al.* (2002) Systems analysis of Ran transport. *Science* 295, 488–491
8 Ribbeck, K. and Gorlich, D. (2001) Kinetic analysis of translocation through nuclear pore complexes. *EMBO J.* 20, 1320–1330
9 Gorlich, D. and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Dev. Biol.* 15, 607–660
10 Lei, E.P. and Silver, P.A. (2002) Protein and RNA export from the nucleus. *Dev. Cell* 2, 261–272
11 Wozniak, R.W. *et al.* (1998) Karyopherins and kissing cousins. *Trends Cell Biol.* 8, 184–188
12 Pemberton, L.F. *et al.* (1998) Transport routes through the nuclear pore complex. *Curr. Opin. Cell Biol.* 10, 392–399
13 Conti, E. and Izaurralde, E. (2001) Nucleocytoplasmic transport enters the atomic age. *Curr. Opin. Cell Biol.* 13, 310–319
14 Simos, G. *et al.* (2002) Nuclear export of tRNA. *Results Probl. Cell Differ.* 35, 115–131
15 Mattaj, I.W. and Englmeier, L. (1998) Nucleocytoplasmic transport: the soluble phase. *Annu. Rev. Biochem.* 67, 265–306
16 Moore, M.S. (1998) Ran and nuclear transport. *J. Biol. Chem.* 273, 22857–22860
17 Cole, C.N. and Hammell, C.M. (1998) Nucleocytoplasmic transport: driving and directing transport. *Curr. Biol.* 8, R368–R372
18 Purcell, E.M. (1977) Life at low Reynolds numbers. *Am. J. Phys.* 45, 3–11
19 Berg, H.C. (1993) *Random Walks in Biology*, Princeton University Press
20 Elbaum, M. (2001) The nuclear pore complex: biochemical machine or Maxwell demon? *C. R. Acad. Sci. Paris, t. 2* Série IV, 861–870
21 Kiseleva, E. *et al.* (1998) Active nuclear pore complexes in Chironomus: visualization of transporter configurations related to mRNP export. *J. Cell Sci.* 111, 223–236
22 Feldherr, C.M. and Akin, D. (1997) The location of the transport gate in the nuclear pore complex. *J. Cell Sci.* 110, 3065–3070
23 Allen, T.D. *et al.* (2000) The nuclear pore complex: mediator of translocation between nucleus and cytoplasm. *J. Cell Sci.* 113, 1651–1659
24 Blobel, G. and Wozniak, R.W. (2000) Proteomics for the pore. *Nature* 403, 835–836
25 Cronshaw, J.M. *et al.* (2002) Proteomic analysis of the mammalian nuclear pore complex. *J. Cell Biol.* 158, 915–927
26 Weis, K. *et al.* (1996) Characterization of the nuclear protein import mechanism using Ran mutants with altered nucleotide binding specificities. *EMBO J.* 15, 7120–7128
27 Schwoebel, E.D. *et al.* (1998) Ran-dependent signal-mediated nuclear import does not require GTP hydrolysis by Ran. *J. Biol. Chem.* 273, 35170–35175
28 Englmeier, L. *et al.* (1999) Receptor-mediated substrate translocation through the nuclear pore complex without nucleotide triphosphate hydrolysis. *Curr. Biol.* 9, 30–41
29 Ribbeck, K. *et al.* (1999) The translocation of transportin–cargo complexes through nuclear pores is independent of both Ran and energy. *Curr. Biol.* 9, 47–50
30 Buss, F. *et al.* (1994) Role of different domains in the self-association of rat nucleoporin p62. *J. Cell Sci.* 107, 631–638
31 Denning, D.P. *et al.* (2003) Disorder in the nuclear pore complex: the FG repeat regions of nucleoporins are natively unfolded. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2450–2455
32 Atkins, P.W. (2001) *The elements of physical chemistry*, W.H. Freeman
33 Bayliss, R. *et al.* (2002) Structural basis for the interaction between NTF2 and nucleoporin FxFG repeats. *EMBO J.* 21, 2843–2853
34 Chaillan-Huntington, C. *et al.* (2000) Dissecting the interactions between NTF2, RanGDP, and the nucleoporin XFXFG repeats. *J. Biol. Chem.* 275, 5874–5879
35 Bednenko, J. *et al.* (2003) Nucleocytoplasmic transport: navigating the channel. *Traffic* 4, 127–135
36 Ribbeck, K. and Gorlich, D. (2002) The permeability barrier of nuclear pore complexes appears to operate via hydrophobic exclusion. *EMBO J.* 21, 2664–2671
37 Dill, K.A. and Bromberg, S. (2003) *Molecular driving forces: statistical thermodynamics in chemistry and biology*, Garland Science
38 Gorlich, D. *et al.* (2003) Characterization of Ran-driven cargo transport and the RanGTPase system by kinetic measurements and computer simulation. *EMBO J.* 22, 1088–1100

39 Ribbeck, K. *et al.* (1998) NTF2 mediates nuclear import of Ran. *EMBO J.* 17, 6587–6598
40 Nachury, M.V. and Weis, K. (1999) The direction of transport through the nuclear pore can be inverted. *Proc. Natl. Acad. Sci. U.S.A.* 96, 9622–9627
41 Hoh, J.H. (1998) Functional protein domains from the thermally driven motion of polypeptide chains: a proposal. *Proteins* 32, 223–228
42 Brown, H.G. and Hoh, J.H. (1997) Entropic exclusion by neurofilament sidearms: a mechanism for maintaining interfilament spacing. *Biochemistry* 36, 15035–15040
43 Mukhopadhyay, R. and Hoh, J.H. (2001) AFM force measurements on microtubule-associated proteins: the projection domain exerts a long-range repulsive force. *FEBS Lett.* 505, 374–378
44 Bickel, T. and Bruinsma, R. (2002) The nuclear pore complex mystery and anomalous diffusion in reversible gels. *Biophys. J.* 83, 3079–3087
45 Shulga, N. and Goldfarb, D.S. (2003) Binding dynamics of structural nucleoporins govern nuclear pore complex permeability and may mediate channel gating. *Mol. Cell. Biol.* 23, 534–542
46 Shulga, N. *et al.* (2000) Yeast nucleoporins involved in passive nuclear envelope permeability. *J. Cell Biol.* 149, 1027–1038
47 Marelli, M. *et al.* (1998) Specific binding of the karyopherin Kap121p to a subunit of the nuclear pore complex containing Nup53p, Nup59p, and Nup170p. *J. Cell Biol.* 143, 1813–1830
48 Ryan, K.J. and Wente, S.R. (2000) The nuclear pore complex: a protein machine bridging the nucleus and cytoplasm. *Curr. Opin. Cell Biol.* 12, 361–371
49 Blobel, G. (1995) Unidirectional and bidirectional protein traffic across membranes. *Cold Spring Harb. Symp. Quant. Biol.* 60, 1–10
50 Akey, C.W. (1990) Visualization of transport-related configurations of the nuclear pore transporter. *Biophys. J.* 58, 341–355
51 Daneholt, B. (2001) Assembly and transport of a premessenger RNP particle. *Proc. Natl. Acad. Sci. U.S.A.* 98, 7012–7017
52 Izaurralde, E. (2002) A novel family of nuclear transport receptors mediates the export of messenger RNA to the cytoplasm. *Eur. J. Cell Biol.* 81, 577–584
53 Stutz, F. and Izaurralde, E. (2003) The interplay of nuclear mRNP assembly, mRNA surveillance and export. *Trends Cell Biol.* 13, 319–327
54 Lyman, S.K. *et al.* (2002) Influence of cargo size on Ran and energy requirements for nuclear protein import. *J. Cell Biol.* 159, 55–67