

The Nuclear Pore Complex: Oily Spaghetti or Gummy Bear?

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In this issue, Frey and Görlich (2007) provide new insight into the selective barrier that controls protein traffic through the nuclear pore complex. They show that a single protein domain of the nuclear pore protein Nsp1 can form a hydrogel that allows highly selective access of nuclear transport receptors and their cargos, but rejects other proteins of similar size.

The nuclear pore complex (NPC) is a remarkable cellular machine. It is responsible for the exchange of proteins, RNAs, and other macromolecules between the cytoplasmic and nuclear compartments in eukaryotic cells, and it does so with extraordinary efficiency and specificity (Tran and Wenthe, 2006). For example, it is estimated that in humans every minute more than one kilogram of material is shuttled across all the NPCs in our body. Despite this staggering flow of mass, the NPC remains highly selective and allows the passage of molecules that are larger than 30–40 kDa only when bound to appropriate transport receptors. But how can a channel accommodate such high rates of transport without losing selectivity? New work by Frey and Görlich (2007) provides evidence that the nuclear pore may function akin to a gummy-like gel and demonstrates that a hydrogel assembled *in vitro* from a single nuclear pore protein domain is capable of mimicking some of the key permeability properties that were previously described for intact NPCs.

With an estimated mass of 40–60 MDa, the NPC is among the largest macromolecular assemblies within the cell. Yet NPCs consist of only about 30 distinct proteins (nucleoporins or Nups), which are all present in multiple copies (Tran and Wenthe, 2006). The NPC channel is open for diffusion of small macromolecules, but beyond 30–40 kDa, nucleocyto-

plasmic transport substrates must contain specific targeting signals, generally referred to as nuclear localization signals (NLS) for nuclear import or nuclear export signals (NES) for nuclear export. These motifs are specifically recognized by soluble nuclear transport receptors that can traverse the NPC either alone or together with their bound cargo. The NPC is thought to be a passive sorting device whose task is to facilitate the selective diffusion of transport receptor-substrate complexes without imparting directionality. The free energy for nucleocytoplasmic transport is largely provided by a steep concentration gradient of the GTPase Ran. This molecule is highly enriched in the nucleus in its GTP-bound form, and GTP hydrolysis by Ran is directly coupled to the import/export cycle (Weis, 2003).

Classes of nuclear pore proteins that contain phenylalanine-glycine (FG) repeats are likely to be critical for the process by which transport receptor-cargo complexes gain selective access to the NPC channel. Roughly one-third of all NPC proteins contain various FG repeat domains. FG repeats are highly unstructured or natively unfolded (Denning et al., 2003) and are thought to line the inner surface of the NPC channel (Tran and Wenthe, 2006; Weis, 2003). All known nuclear transport receptors can bind to FG-containing Nups, and interactions between transport receptors and FG repeats are essen-

tial for translocation through the NPC (Weis, 2003). However, the biophysical details of how these FG filaments contribute to the selective permeability of the NPC have been a matter of debate.

Several models have been proposed to explain the gating behavior of the NPC (Figure 1). The “virtual gating” model views the NPC as a catalyst that can lower the activation energy for the translocation process (Rout et al., 2003). The barrier for large molecules to cross the NPC is normally high because they have to enter a narrow NPC tunnel, potentially crowded by FG repeats, leading to a significant decrease in their entropy. Binding of transport receptors to FG repeats would overcome this entropic barrier and would provide a significant kinetic advantage for cargos that are bound to transport receptors. Because the selectivity that is achieved by this mechanism does not rely on a mechanical barrier the term “virtual gating” was introduced to describe this model (Rout et al., 2003). In a similar way, the “oily spaghetti” model suggests that long hydrophobic FG repeats normally occlude the NPC channel but can be pushed aside by receptor-cargo complexes (Macara, 2001). More recently, atomic force microscopy was used to show that certain FG repeats can indeed extend or collapse, suggesting that they act like polymer brushes that could contribute to an entropic barrier at the NPC (Lim et al., 2006).

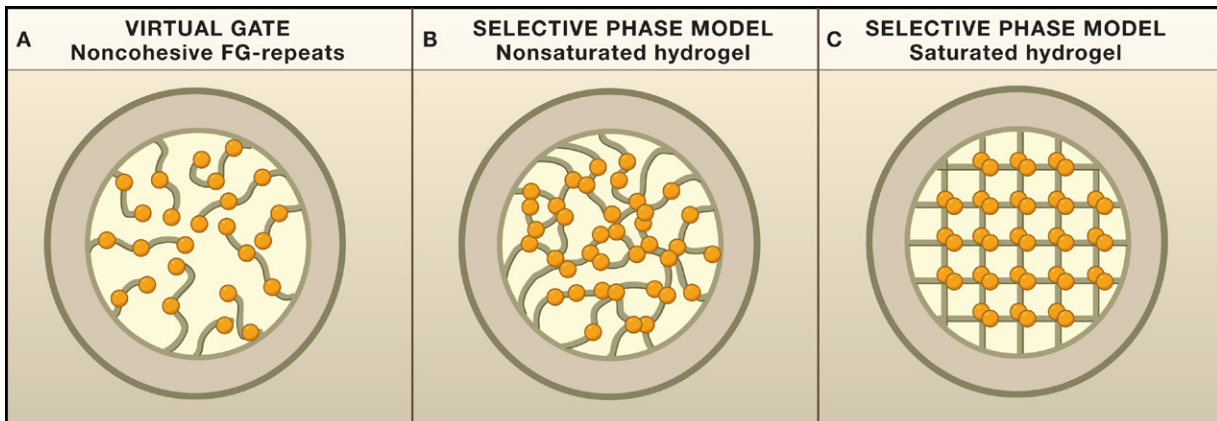


Figure 1. Establishing Selectivity at the Nuclear Pore Complex

Different models for how FG-repeat proteins establish selectivity at the nuclear pore complex (NPC) are shown.

(A) Unstructured noncohesive FG-repeat-containing filaments fill the NPC channel to entropically exclude cargo not bound by nuclear transport receptors. FG repeats (orange) do not necessarily mechanically restrict the access of cargo, but binding of transport receptors to FG repeats would significantly speed up translocation, thus establishing a “virtual gate” (Rout et al., 2003). This entropic gate may be located at the mouth of the channel in the form of FG bristles (Rout et al., 2003) or polymer brushes (Lim et al., 2006), or, alternatively it could fill the whole channel like a bowl of “oily spaghetti” (Macara, 2001).

(B) The “selective phase” model (Ribbeck and Görlich, 2001) suggests that FG filaments interact with each other to form a selective three-dimensional sieve that excludes large molecules.

(C) Frey and Görlich (2007) propose the formation of a “saturated hydrogel” within the NPC, in which all the FG domains engage in a maximum number of interactions to form a highly ordered mesh with very even pore size. In both models (B) and (C), transport receptors are thought to dissolve the FG mesh and thus catalyze the entry and translocation of cargo through the NPC channel.

These kinetic models are in contrast to the “selective phase” model (Ribbeck and Görlich, 2001) that proposes the formation of a sieve-like meshwork within the NPC through interactions between FG-containing repeats (Figures 1B and 1C). Here, the size of the FG mesh determines the upper limits of the diffusion gate and mechanically restricts access of large molecules. However, the binding of transport receptors to FG repeats is proposed to dissolve the FG-FG network, and therefore transport receptors partition into this specific phase, which would explain how large receptor-cargo complexes gain exclusive access to the NPC. The selective phase model makes two key predictions. First, it requires that FG repeats form interactions, and second, it predicts the existence of a selective phase within the NPC channel.

Interestingly, it was recently shown that the FG-repeat domain of the yeast nucleoporin Nsp1 can form a hydrogel-like structure *in vitro* that requires hydrophobic interactions between aromatic rings (Frey et al., 2006). Furthermore, various FG-repeat domains can form weak and reversible homo- and heterotypic interac-

tions *in vitro* and *in vivo* (Patel et al., 2007). However, interactions were only detected between FG domains that are located in the central channel of the NPC. This led to the proposal of a two-gate mechanism that combines a central gate, established by a “selective phase” of interacting FG repeats within the NPC channel and a “virtual gate,” formed by noncohesive FG filaments located at the periphery of the NPC (Patel et al., 2007).

These new studies firmly established that FG repeats can interact, but experimental evidence had been lacking for any of the proposed models to test whether the suggested mechanisms could provide sufficient selectivity to explain the permeability behavior of the NPC. In an astonishing set of experiments, Frey and Görlich now demonstrate that a hydrogel formed by the FG-repeat domain of Nsp1 displays selective properties that are reminiscent of the gating behavior of NPCs (Frey and Görlich, 2007). FG-hydrogels were assembled *in vitro*, and gel entry and diffusion rates of several proteins were examined by fluorescence microscopy. Remarkably, the influx of various nuclear transport receptors of the

importin β family into the Nsp1 FG-hydrogel was $\sim 1000\times$ faster than the entry of a control protein. Access of a model cargo bound to importin β was accelerated by more than $20,000\times$ when compared to free cargo alone. Furthermore, the measurements of intra-gel diffusion rates matched up with recently published rates for NPC translocation derived from single molecule experiments (Kubitscheck et al., 2005; Yang et al., 2004).

Although these results are truly remarkable and highly suggestive, the question remains whether such a hydrogel exists within the NPC and whether these results reflect the *in vivo* physiology of nuclear transport. Interestingly, not every FG-hydrogel displays selectivity, and an Nsp1 FG-hydrogel that was formed according to previously published conditions (Frey et al., 2006) allowed equal entry of all proteins and did not discriminate between nuclear transport receptors and cargo alone (Frey and Görlich, 2007). In order to achieve selective permeability, the total FG concentration within the gel had to be raised above 50 mM. This led the authors to introduce the concept of the saturated hydrogel, in which all the FG

repeats have to extend completely and undergo a maximum number of interactions. This would allow the formation of a highly ordered mesh required to establish an efficient permeability barrier (Figure 1C). Despite the fact that the local concentration of FG repeats within the NPC may be high enough to achieve “saturation,” it is hard to imagine how such a perfect FG network could be established in vivo, especially given that newly synthesized FG repeats would most likely immediately curl up and form intramolecular FG bridges. Indeed, in vitro gel formation can only be induced from lyophilized proteins under extreme pH and salt conditions. In order to overcome this conceptual problem, the authors suggest that nuclear transport receptors could act as chaperones. This would help to prevent intramolecular FG interactions after synthesis, with

mesh formation being catalyzed once the nuclear pore protein reaches the NPC (Frey and Görlich, 2007).

Saturated or not, the in vivo evidence for the existence of a FG-hydrogel within the NPC (Frey et al., 2006) remains weak. Under more physiological conditions, the FG domain of Nsp1 formed neither homo- nor heterotypic interactions with other FG nucleoporins (Patel et al., 2007). Therefore, the final answer to the question of whether the NPC looks more like a bowl of spaghetti or behaves like a gummy bear almost certainly requires additional structural and biophysical studies most likely paired with high-resolution single molecule experiments.

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GRASPing Unconventional Secretion

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GRASP proteins associate with the Golgi apparatus and have been implicated in the stacking of Golgi cisternae, vesicle tethering, and mitotic progression, but their specific functions are unclear. In this issue, Kinseth et al. (2007) show unexpectedly that a GRASP homolog is required for an unconventional secretory pathway that bypasses the usual route for Golgi-dependent membrane traffic.

The form and function of the Golgi apparatus are tightly linked. Proteins destined for secretion are modified in the stacked Golgi cisternae, yet the mechanisms that generate this elaborate structure remain mysterious. An in vitro assay to identify factors involved in Golgi assembly led to the identification of mammalian GRASP65, which became the founding member of the GRASP (Golgi reassembly stacking protein) family of peripheral mem-

brane proteins (Lowe and Barr, 2007). GRASP65 localizes to pre-Golgi and early Golgi compartments, and GRASP55—the other GRASP in mammalian cells—localizes to the medial Golgi. GRASPs are widely conserved in eukaryotes. For example, the fruit fly *Drosophila melanogaster* and the yeast *Saccharomyces cerevisiae* contain GRASP65 orthologs called dGRASP and Grh1, respectively (Konody et al., 2005; Behnia et al., 2007).

GRASPs are important for the normal structure and operation of the Golgi, but little is known about their biochemical activity. To address this question, Kinseth et al. (2007) examined the GRASP homolog in the slime mold *Dictyostelium discoideum*.

Earlier studies in several organisms analyzed the role of GRASPs in Golgi architecture. Mammalian GRASP65 forms a complex with its partner protein GM130, which interacts in turn with