CELL BIOLOGY

Nuclear Pore Complex Models Gel

Two views are given on the elastic structure of pores in the cell's nuclear membrane, which allows the exchange of materials between the nucleus and the cytoplasm.

Brian Burke

In eukaryotic cells, the boundary between the nucleus and cytoplasm is defined by a membranous organelle, the nuclear envelope. Trafficking of macromolecules back and forth across this envelope occurs through nuclear pore complexes (*1*). A vertebrate somatic cell typically contains between 1000 and 10,000 such pore complexes. Small molecules can pass through these pores unimpeded; larger molecules (30- to 40-kD proteins) must associate with soluble nuclear transport receptors and be escorted through the central channel of the pore. We know much about the structure of the nuclear pore complex and the role of soluble components in nucleocytoplasmic transport, but the mechanics of translocation through the nuclear pore complex have been debated. On page 815 of this issue, Frey *et al*. (*2*) provide evidence for the existence of a flexible sieve that spans the pore, creating a selective permeability barrier.

Nuclear pore complexes are composed of multiple copies of about 30 different protein subunits (nucleoporins or nups). About one-third of these nups contain FG domains featuring arrays of the hydrophobic peptide repeats FG, GLFG, or FXFG (F, Phe; G, Gly; L, Leu; X, any amino acid). FG domains are thought to be natively unfolded, adopting extended, flexible conformations. They are also considered to have a central role in nuclear pore complex function, because FG repeats bear binding sites for nuclear transport receptors.

But how exactly do FG nups mediate nucleocytoplasmic transport? Two mechanistic models have dominated this discussion. The first, proposed by Rout *et al*. (*3*), invokes the concept of virtual gating. In this scheme, FG nups increase the residence time of transport complexes in the central aperture of the pore by binding to nuclear transport receptors. In this way, FG nups facilitate diffusion of transport complexes into the central channel. Conversely, because FG domains are flexible and largely unstructured, they limit available space in the nuclear pore complex near-field, thus restricting access of nontransport substrates to the nuclear pore complex. Recently, Aebi and colleagues (*4*) used atomic force microscopy to study the FG domain of vertebrate Nup153 immobilized on gold nanodots. They concluded that FG domains cluster and form a "polymer brush" that could indeed display the type of exclusionary function that is key to virtual gating. A more recent proposal based on constrained diffusion within the nuclear pore complex central channel also highlights aspects of virtual gating (*5*).

Ribbeck and Görlich (*6*) proposed an alternative model in which FG domains within the central channel of the pore complex interact through FG repeats to form a protein meshwork, essentially forming a separate hydrophobic phase. Transport complexes can partition into this phase because of their capacity to bind to the FG repeats, thereby locally perturbing FG domain interactions. Proteins incapable of binding FG repeats are excluded from this hydrophobic phase. Small molecules and proteins below the size exclusion limit pass through the interstices of such a meshwork, independent of transport receptors. An earlier proposal by Macara and colleagues, called the "oily spaghetti" model (*7*), presents some features of the selective phase concept and similarly underscores the hydrophobic nature of the unstructured FG domains.

An important difference between the virtual gating and selective phase models concerns the interaction between FG repeats.

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MATERIALS SCIENCE

Polymers in the Pore

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The nuclear pore complex may be nature's ultimate analytical chemist. Seated at the gateway between the nucleus and cytoplasm in eukaryotic cells, it distinguishes a mixed solution of macromolecules by their chemical identity, all the while remaining open to diffusive passage of water, ions, metabolites, and other small solutes. From a physical point of view, it is a fascinating machine. Frey *et al.*, on page 815 in this issue, explore the unlikely talent of this specially tuned barrier (*1*).

Up to a size cutoff of a few nanometers, the nuclear pore acts as a simple sieve. Beyond ~40 kD, most proteins and protein complexes are unable to cross it on their own. Nuclear transport receptors may usher such larger cargoes specifically across the pore. Ironically, a midsize protein must recruit a large receptor to pass through a narrow channel. Clearly there is something special in the recognition of transport receptors by whatever makes up the sieve within the pore. Attention has focused on repeat motifs of phenylalanine-glycine (FG) that are common among constituent proteins of the nuclear pore itself. The FG repeats do indeed interact with nuclear transport receptors. Moreover, these domains tend to be natively unfolded polypeptides, so they are presumed to swell into the central channel of the pore. From here, the key questions are essentially of polymer physics and chemistry.

What is the nature of this FG-repeat network? It was proposed to form a hydrogel, cross-linked by hydrophobic interactions between the phenylalanines (*2*). Frey *et al*. show that FG repeats can indeed form a free-standing gel, and they measure elasticity comparable to 0.4% agarose. They show further that mobility of fluorescently labeled FG peptides is low in the gel, consistent with cross-linking among them. Mutating the phenylalanines to serines results in both loss of gel stiffness and a higher mobility of the polymers.

A natural scale for the nuclear pore sieve is then simply the mesh size of the gel. Mobility of

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The former model predicts mobile FG domains, implying minimal interactions. This prediction is supported by the atomic force microscopy observations of the Nup153 FG domain. The latter model requires more stable interactions between FG domains to establish a meshwork. Frey *et al*. have found that the FG domain of yeast Nsp1p will form a mechanically stable elastic hydrogel in vitro, featuring hydrophobic interactions between FG repeats. These Nsp1p hydrogels can even incorporate the FG domains of other FG nups. Hydrogel formation is absolutely dependent on the FG repeats because it is abolished by substitution of S (Ser) for every F within the Nsp1p FG domain (Nsp1pF→S).

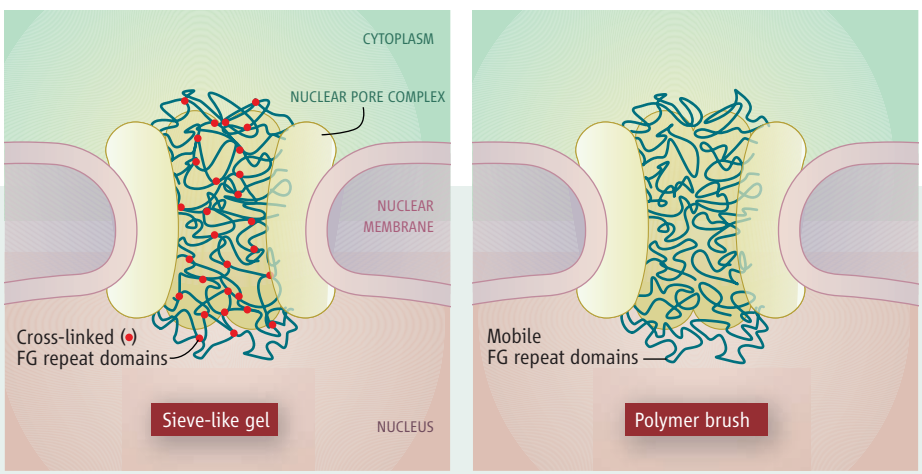
Wente and colleagues had previously demonstrated redundancy in the FG domains of yeast nups (*8*). In particular, FG domains of nups that are distributed asymmetrically at the nucleoplasmic or cytoplasmic face of the nuclear pore complex appear dispensable. Indeed, deletion of the FG domain of Nsp1p, which localizes to the cytoplasmic side of the yeast pore complex, has little or no effect on viability. However, Frey *et al*. now show that substitution of Nsp1p by Nsp1pF→S is lethal in yeast and that this lethality cannot be attributed to inability to bind nuclear transport receptors. They suggest that Nsp1pF→S perturbs the hydrophobic characteristics of the FG phase within the nuclear pore complex, leading to loss of pore functionality.

This new study provides some compelling evidence for the selective phase model. Nonetheless, questions still remain. For instance, the FG domains of certain vertebrate nups are extensively modified with O-linked *N*-acetylglucosamine (*9*). How this might affect interactions between FG domains remains to be seen. Furthermore, not all FG domains may be equivalent. A distinct possibility is that while certain nups may contribute to a selective FG phase within the core of the pore complex, others at the periphery might behave more like a virtual gate. As biophysical and genetic approaches are brought to bear on the problem, the answer may be close at hand.

References

1. E. J. Tran, S. R. Wente, *Cell* **125**, 1041 (2006).
2. S. Frey, R. P. Richter, D. Görlich, *Science* **314**, 815 (2006).
3. M. P. Rout, J. D. Aitchison, M. O. Magnasco, B. T. Chait, *Trends Cell Biol.* **13**, 622 (2003).
4. R. Y. Lim *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **103**, 9512 (2006).
5. R. Peters, *Traffic* **6**, 421 (2005).
6. K. Ribbeck, D. Görlich, *EMBO J.* **21**, 2664 (2002).
7. I. G. Macara, *Microbiol. Mol. Biol. Rev.* **65**, 570 (2001).
8. L. A. Strawn, T. Shen, N. Shulga, D. S. Goldfarb, S. R. Wente, *Nat. Cell Biol.* **6**, 197 (2004).
9. G. D. Holt *et al*., *J. Cell Biol.* **104**, 1157 (1987).

10.1126/science.1135739



Pore gel. Alternate views on the polymeric state of natively disordered FG-repeat domains swelling into the transport channel of the nuclear pore.

nuclear transport receptors is explained in this scheme by their ability to replace the labile bonds between the polymers with links to themselves. In other words, they dissolve into the gel. Partitioning of transport receptors between the gel and surrounding aqueous phases allows for molecular exchange and transport. Just as hydrophobic moieties cross lipid bilayers much faster than hydrophilic ones, specific hydrophobic interactions between the FG proteins and the transport receptors allow the receptors to cross the FG sieve as a "selective phase" barrier (*2*). Frey *et al.* show that mutating the phenylalanines to tyrosines suppresses the interaction with transport receptors, but the gel state is retained, showing that the two features are independent.

An alternate view holds that FG repeats could form a network of unlinked polymers whose thermally activated undulations create a zone of "entropic exclusion" (*3*). The principle is similar to stabilization of colloids by capping their surfaces with long-chain molecules. The entropic penalty in collapsing these chains prevents aggregation of neighboring particles. By transiently attaching to the FG polypeptides, perhaps at multiple points, transport receptors could circumvent this exclusion. Conceptually, this model is inspired by weak repulsive forces between neurofilaments, a cytoskeletal structure that gives mechanical strength to axons and dendrites in neurons (*4*). Indeed, Lim *et al.* (*5*) recently found that end-anchored FG repeats show entropy-dominated elastic properties of a "polymer brush" (*6*). The force measured in compressing the brush grows exponentially as the gap is closed. At least in vitro, the FG-repeat networks tested can take both proposed forms.

Probing the mobility of nuclear transport receptors in well-defined FG gels or brushes will require further analyses, catching up in a way with single-molecule studies made recently in native nuclear pores (*7*, *8*). Whichever mechanical model of its sieve turns out to be more relevant in the cellular context, understanding the polymer physics of the nuclear pore may inspire novel biomimetic materials or nanotechnological devices to corral specific macromolecules from a mixed solution. Enantiomeric separation by antibody (*9*) or polypeptide-lined (*10*) membrane pores could make an interesting start in this direction. A long road lies ahead, though, until materials science can match the exquisite single-residue sensitivity of the nuclear pore and its transport receptors.

References

1. S. Frey, R. P. Richter, D. Görlich, *Science* **314**, 815 (2006).
2. K. Ribbeck, D. Görlich, *EMBO J.* **20**, 1320 (2001).
3. M. P. Rout *et al*., *J. Cell Biol.* **148**, 635 (2000).
4. H. G. Brown, J. H. Hoh, *Biochemistry* **36**, 15035 (1997).
5. R. Y. H. Lim *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **103**, 9512 (2006).
6. H. J. Taunton, C. Toprakcioglu, L. Fetters, J. Klein, *Macromolecules* **23**, 571 (1990).
7. U. Kubitscheck *et al*., *J. Cell Biol.* **168**, 233 (2005).
8. W. Yang, S. M. Musser, *J. Cell Biol.* **174**, 951 (2006).
9. S. B. Lee *et al*., *Science* **296**, 2198 (2002).
10. N. H. Lee, C. W. Frank, *Polymer* **43**, 6255 (2002).

10.1126/science.1135924