Physica A 404 (2014) 65–78

Contents lists available at ScienceDirect

Physica A

journal homepage: www.elsevier.com/locate/physa

Diffusion in an elastic medium: A model for macromolecule transport across the nuclear pore complex

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HIGHLIGHTS

- An analytical theory for the transport of macromolecules through nuclear pore complex.
- Valid for brush as well as gel picture of nuclear pore complex.
- Enhancement of diffusion due to fluctuations in elastic environment.
- Selectivity in protein transport arises as a result of the competition between elastic, deformation and contact energy.

ARTICLE INFO

Article history:
Received 24 November 2013
Received in revised form 17 February 2014
Available online 28 February 2014

Keywords:
Nuclear pore complex
Diffusion of macromolecules
Brush vs gel picture of nuclear pore complex
Enhancement in diffusion

ABSTRACT

Nuclear pore complexes (NPCs) are very selective filters that sit on the membrane of the nucleus and monitor the transport between the cytoplasm and the nucleoplasm. For the central plug of NPC two models have been suggested in the literature. The first suggests that the plug is a reversible hydrogel while the other suggests that it is a polymer brush. Here we propose a model for the transport of a protein through the plug, which is general enough to cover both the models. The protein stretches the plug and creates a local deformation, which together with the protein, we refer to as the bubble. We start with the free energy for creation of the bubble and consider its motion within the plug. The relevant coordinate is the center of the bubble which executes random walk. We find that for faster relaxation of the gel, the diffusion of the bubble is greater.

1. Introduction

The nucleus in a eukaryotic cell is perforated with a large number of pores [1–12]. Each such pore has a selective filter, referred to as the nuclear pore complex (NPC) [1]. The NPC regulates the import and export of proteins. The transport through the NPC can be passive or facilitated. Passive transport is non-specific and takes place by ordinary diffusion. Colloidal gold particles with radius up to 6–7 nm, and generic proteins up to 30 kDa in mass, pass efficiently through the NPC in this way [13] but bigger particles are not allowed to go in this mode. In contrast, facilitated translocation allows the passage of objects as large as several megadaltons. The transport is catalyzed by nuclear transport receptors (NTR), which are rich in hydrophobic units. A protein having an appropriate amino acid sequence, referred to as the nuclear localization signal (NLS) can form a complex with NTR [3] and then be transported selectively, even if its size exceeds 30 kDa. Gold particles of up to 32–36 nm in diameter are able to pass through some NPC if they are coated with nucleoplasmin–importin complexes [4]. NTRs allow the recognized cargos to move at rates 100–1000 times faster than inert molecules of comparable size. The

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http://dx.doi.org/10.1016/j.physa.2014.02.059
0378-4371/

transport is driven by the active release of the cargo in the destination compartment, which consumes RanGTP (a small protein Ran bound to guanosine triphosphate), but the actual transport within the NPC is believed to be diffusive [14,15].

The rigid scaffold of the NPC is built from nucleoporins. About 12%–20% of the mass of the NPC is made up of the FG (phenylalanine glycine)—repeat domains [16,17]. There are different varieties of FG repeat units, but they are all hydrophobic. They are separated by linkers of variable sequence and length, which can be extremely hydrophilic. These hydrophobic domains play a crucial role in the transport of NTR-cargo complexes. Nucleoporins are unfolded in their native state, and have no specific secondary structure. Scanning force microscopy studies on Nup 153, physically adsorbed on mica show that the molecule is 200 nm long and has a cross sectional width of 0.4 nm [6].

Kinetic studies have shown that the mass flow through NPC is of the order of 100 MDa per second, and the rate is of the order of 10^3 translocations per second [2,18]. How does NPC achieve such a rate with high degree of selectivity? The mechanism of transport is not understood at all [4]. There is evidence that it involves interaction between the FG-repeats and the NTRs. The interaction involves the phenylalanine ring of the FG and the hydrophobic residues on the surface of NTR. The interaction is predominantly hydrophobic, but other interactions (H-bonding, electrostatic interactions) may also play an important role [19–21]. Different models have been proposed for the protein [4] and RNA transport [10] through NPC. These include virtual gating [16], affinity gradient [22], reduction in dimensionality [5], selective phase [2] and very recently proposed hybrid model [23]. Of them, the most important are the Gel model [24,25] and the Brush model [25,26]. Both of them seem to have support from experiments and have been the subject of a number of papers. The final conclusion on this is yet to be arrived at. As a result, the passage of proteins through the NPC has attracted considerable experimental [2,14, 15,27–29] and theoretical attention [19,30–37].

One of the two competing models is the gel model, due to Ribbeck and Görlich (RG) [2] in which the nucleoporins are assumed to make hydrophobic contacts, resulting in a network, which behaves like a gel. The mesh size of the network is of the order of 5–7 nm, allowing Au nanoparticles or macromolecules of this size to pass through easily, and preventing bigger ones, unless they have a chemical affinity for the network. This model was theoretically analyzed by Bickel and Brunisma (BB) [30] who pointed out that the network lowers the rate of diffusion, but also that noise from the network can be expected to enhance the diffusion. More recently Nielsen et al. [34] have carried out Monte Carlo simulation to explore the physical mechanism of macromolecule transport across the nuclear pore complex. Brownian dynamics simulations of the model were done by Kustanovich and Rabin [31]. They find that the efficiency of the transport increases as the particle is able to make more contacts. Considerable amount of computational work has been done by the group of Schulten [19,32,33] to explore the binding sites on the proteins that are able to go across the nuclear pore. On the experimental side, single molecule fluorescence microscopy by Yang et al. [14] showed that the protein executes random walk inside NPC. The FG-repeat domains of nucleoporins (Nups) which are within the plug of the NPC have been shown to bind to each other by hydrophobic interactions both in vivo and in vitro [27]. In support of the model, Frey and Görlich [24] showed that FG repeats of the nucleoporins can cause them to form a gel. Further, fluorescently labeled FG peptides were shown to have low mobility in the gel, indicating the presence of a network. Continuing the work, Frey et al. [1,24,38] demonstrated that a hydrogel is formed by the FG-repeat domain of Nsp1 (a nucleoporin) and that at sufficient concentrations, the gel was shown to display properties very similar to the gating behavior that is observed for the NPCs. The measured intra-gel diffusion rates [1] of the translocating species matched up with measured rates [14] for NPC translocation, thus providing evidence for the suggestion that the plug is a gel.

However, there is equally strong evidence for a model which suggests the FG rich nucleoporins to form a polymer brush [26,39]. In the brush model, the FG contacts between the different nucleoporins is not considered to be important, and there does not exist a network that has to be broken, for the cargo to get in. The cargo is prevented from entering by the entropic repulsion of the brush. In interesting experiments, the FG domain of one human Nup (Nup 153, which is anchored at the nuclear basket) was tethered to isolated Au nanodots and studied using force microscopy. They were found to exert an exponentially decaying repulsive force characteristic of a brush [16,26]. It has been suggested [6] that about 10 molecules of FG-nucleoporins is enough to maintain the entropic barrier in native NPCs. Alber et al. [7,8] suggest that the permeability barrier is formed by large number of proteins which have disordered regions.

In the following we study a minimalistic model for the transport in the NPC. The model is very general and in our opinion, captures the essential physics of the problem. The plug is modeled as an elastic continuum, which is distorted by the entry of the cargo-NTR complex. The distortion energy is compensated by the contact of the surface of the complex with the hydrophobic part of the plug. With suitable values for the parameters, it can be used to model a gel or brush. The model does not take curvature energy where the molecular energy is accounted for, in consideration as is usually incorporated in the models for membranes [40,41]. It will be interesting to investigate how the incorporation of curvature energy in the model affects the results. This can only be possible in a detailed computer simulation which will be a subject of our future study.

1.1. Discrete model

To make the ideas clear, we start with a discrete model and then switch over to the continuum version, as that is easier to analyze. We imagine the NPC to have cylindrical shape, with a radius R_p and length L. The total surface area of the cylindrical pore will be denoted by $\mathcal{A} = 2\pi R_p L$. Proteins are imagined to be attached to the inner surface of the pore, with a number density per unit length equal to ρ, so that the total number of proteins attached to the surface will be $\mathcal{N} = \mathcal{A}\rho^2$. These proteins are assumed to form a random network, in which hydrophobic contacts are made, and they fill up the space within the pore.