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Why RNA and Protein Chains Are Linear and Not Colliding inside Nuclear Pores

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It is known that RNA and protein chains in eukaryotic cells are all linear while passing through small nuclear pores. Moreover, when a cell is active, macromolecules pass through nuclear pores at a rate of $\sim 10^3$ chains/s, roughly one chain per pore per second. These facts raise two questions: (A) whether linear RNA/protein chains are accidental or a result of natural selection with some underlining physical principles and (B) how these chains are able to avoid collisions with one another at such a rate of traffic and within so confined a space. On the basis of our previous studies of vibration frequency of a thin-walled porous hollow sphere filled with liquid in dispersion and of the translocation of polymer chains with different topologies through a cylindrical pore, we hypothesize and link these two questions to thermally agitated vibrations (breathing motions) of the nuclear membrane, as schematically shown in Figure 1.

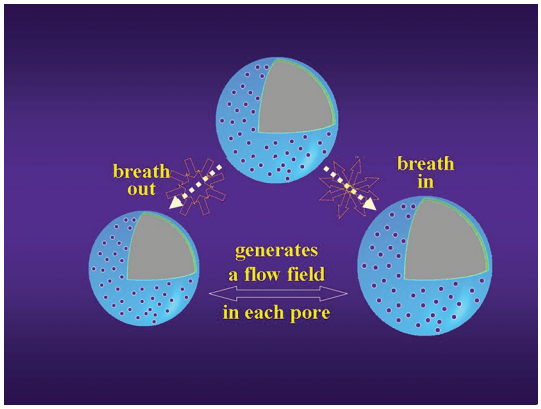

Figure 1. Schematic of thermally agitated breathing-out and breathing-in of a porous hollow sphere with a thin wall and filled with liquid in dispersion.

Note that in Figure 1 we have purposely exaggerated the size change induced by the thermal fluctuation. In reality, taking a polymer microgel as an example, the thermal energy does not agitate and excite the entire microgel to undergo the internal motions (breathing) but only a small portion of swollen gel network or a subchain between two cross-linking points or even a segment of the subchain, depending on the cross-linking density and the chain rigidity, to continuously undergo the Brownian motions. Further, taking a hollow sphere made of a thin porous polymer gel film as an example, the thermal energy does not excite the entire hollow sphere to undergo the internal motions but only a portion of the gel film with a dimension of ~50 nm to undergo the breathing modes, like a surface wave. Therefore, it is impossible that the thermal energy would excite the entire nuclear membrane to breath with visible and macroscopic amplitude. Actually, instead of the shrinking/swelling, it should be only the surface wiggling, like a surface wave.

We hypothesize that the "breathing" motion of each pore either sucks in or pushes out a small amount of liquid that generates a flow with a rate (q) through each pore, which can drive a linear protein or RNA chain to pass through it. For each pore at any given moment, the flow can only be either in or out and in only one single direction. If this passive RNA- and protein-driving process exists on top of all previously described/studied active mechanisms, we will have explained why there are no RNA and protein collisions inside nuclear pores. Even if our hypothesis is correct, there is the question of whether a breathing-generated flow field is sufficiently strong to suck or push a protein/RNA chain in or out of a small nuclear pore. To tackle this, we start with the study of chain confinement and translocation in and through a small pore, which has been of academic interest for a long time and has various applications, such as size exclusion chromatography, ultrafiltration, and controlled release, to name a few. Casassa and Tagami[1] and Edwards and Freed[2] first theoretically studied the confinement of polymer chains using classical eigenfunction expansion methods for solving diffusion equations in as early as 1969. This was followed later by de Gennes[4] and Pincus,[5] who, using a Rouse model,[3] predicted that linear chains in a dilute solution can undergo a first-order coil-to-stretch transition in an elongation flow field at a critical (minimum) flow rate (q_c), which enables a chain to pass through a cylindrical tube much smaller than its coiled size if the hydrodynamic drag force is sufficiently strong. By approximating the confined chain as a string of nondraining hard spheres (blobs), they showed that q_c is independent of both the chain length and the tube radius (R), more specifically $q_c \simeq k_B T/12\pi\eta$, and only related to the absolute temperature (T) and the solution viscosity (η), where k_B is the Boltzmann constant and each confined space occupied by a subchain inside the pore is treated as a small cylinder with a volume of $2\pi R^3$.

Although the prediction was made in the early 1970s, it had been left unconfirmed experimentally until we recently experimentally found that the coil-to-stretch transition is the first order with a critical (minimum) flow rate ($\sim 10^{-15}$–10^{-16} cm^3/s). Such a critical flow rate is indeed *independent of the chain length*; namely, linear chains with different lengths are able

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to pass a cylindrical pore much smaller than their coiled sizes as long as $q \geq q_c$ but retained when $q < q_c$, *an all-or-none process*. However, our measured q_c is $\sim 10^2-10^3$ times smaller than the predicted value, depending on the solvent quality, and reciprocally proportional to the pore size.[6,7] These discrepancies were later attributed to the previous wrong assumption of treating each confined subchain as a hard sphere. After considering the hydrodynamic draining, we have quantitatively and satisfactorily explained why our measured critical flow rate is much smaller and decreases as the pore becomes larger.[8,9]

Further, we also theoretically derived and experimentally proved that polymer chains with a star shape have a higher q_c than linear ones and q_c quickly increases with the arm number.[10] For other topologies, de Gennes[11] predicted that a branched chain has a much higher q_c than its linear counterpart. Moreover, its q_c is dependent not only on the length of subchains between two neighboring branching points but also on the overall molar mass. Our recent experimental results have demonstrated such predictions (unpublished).

All of these experimental and theoretical results lead us to conjecture that it is not an accident that RNA and protein chains are linear. If they were branched, the critical flow rate for them to pass through nuclear pores would be varying for different branching structures and different molar masses, but the thermally agitated motions of a nuclear membrane are passive such that the induced flow rate cannot be automatically adjusted for branched chains with different structures. Only when RNA and proteins are linear does the flow-induced translocation through each nuclear pore become independent of the chain length. At this point, an attentive reader might argue that RNA molecules are "branched" chains if we consider their secondary structures hold together by hydrogen bonds, which is true. However, the passing through the pore is not necessary involving the breaking of all the hydrogen bonds inside individual modules (α-helix and β-sheet) to stretch the entire chain because the nuclear pore has a size larger than the diameters of these modules. An attentive reader might also further ask "assuming that your hypothesis is true, do these "breathing" motions induce a flow rate sufficiently higher than your measured q_c? Does this flow rate depend on the radius (r) of a nucleus?"

Quantitatively, the breathing frequency (f) is reciprocally proportional to the nucleus radius (r_n), i.e., $f = m/r_n$, where m is a constant, increasing with the membrane elastic modulus but decreasing with the medium viscosity, and the nucleus volume (V) is $4\pi r_n^3/3$. Let us assume that the breathing-induced nucleus volume change is $\Delta V = 4\pi r_n^2 \Delta r_n$ and the average number (n) of pores on each nucleus is $\sigma \cdot 4\pi r_n^2$ with σ being the average surface density of nuclear pores, and the breathing-induced flow rate per nuclear pore is

$$q = \frac{\Delta V}{n} f = \frac{3V}{n} \frac{\Delta r_n}{r_n} f = \frac{m}{\sigma} \frac{\Delta r_n}{r_n} \tag{1}$$

This shows that the generated flow rate depends only on the relative size change, not on the nucleus size, which is important to our hypothesis because nuclei do have different sizes.

Our previous study revealed that a porous hollow polymer sphere with a wall thickness of ~7 nm vibrates ("breathes") in an organic liquid with a frequency of $\sim 10^5$ Hz and $m \sim 1-2$ cm/s.[12] Assuming that $\Delta r_n/r_n$ is as small as ~1% and replacing V and n with their typical values, we will still have $q > q_c$ even if f is as low as 10^2 Hz. This estimation clearly shows that the thermally agitated motions of the nuclear membrane can generate a sufficiently high flow field to drive linear RNA and proteins through nuclear pores passively, *irrespective of their chain lengths*. Note that it is not clear whether individual RNA and protein chains pass through the nuclear pore as a folded conformation or as a stretched one at this moment because the narrowest part of the pore only has a diameter of 9−26 nm and a typical mRNA has ~1000 nucleotides with a size of 50−100 nm.

The next question is whether each RNA or protein chain has a sufficiently long time to pass through a nuclear pore under such a thermal agitation-induced flow. It has been known that each nuclear pore has an opening of ~100 nm at its entrance and a total length of ~200 nm, but its middle part (spokes) is much narrower with a diameter of only ~9−26 nm and a length of ~50 nm. We can estimate the time of a linear RNA or protein chain to pass a nuclear pore as follows:

$$t = \frac{l}{v} = \frac{l\pi r_p^2}{q} = \frac{l\pi r_p^2 n}{f\Delta V} = \frac{l r_p^2 n}{4 r_n^2 \Delta r} \frac{1}{f} \tag{2}$$

where l is the pore length and v is the flow velocity, defined as q divided by the cross-section area of the pore (πr^2), r_p is the radius of the nuclear pore, and q is given in eq 1. Replacing l, r_p, r_n, and n in eq 2 with their typical values, we know that if $\Delta r > 1$ nm, t will be shorter than the time duration of each "breath" ($1/f$). Because the nuclear double layer membrane has a thickness of ~6 nm, it is reasonable to assume that it will be able to fluctuate over a linear distance longer than 1 nm. Therefore, eq 2 shows that under our hypothesis there is sufficient time for a linear RNA or protein chain to pass through a nuclear pore completely without being driven back and forth inside a nuclear pore by the breathing motions.

Furthermore, considering that the RNA and protein translocation frequency per nuclear pore is ~1 Hz, we realize that only one out of many "breathing" motions actually drive a protein or RNA chain into or out of each nuclear pore. In reality, f should be different from 10^5 Hz because the nuclear membrane with its inner protein meshwork is more rigid than our previously studied porous polymer membranes and its inside and outside liquids are more viscous than our previously used organic solvent. By reasonably assuming its elastic modulus and surrounding viscosities on the basis of our macromolecular knowledge, we are able to estimate that f is still much higher than 1 Hz. Namely, a linear RNA or protein chain has sufficient time to pass through a nuclear pore under a sufficiently strong "breathing"-induced velocity gradient at its entrance as long as a RNA or protein chain is actively brought close to or into a nuclear pore, although most of the time the "breathing" motions derive no RNA or protein chains.

In summary, on top of all existing active biological processes, we hypothesize that a passive process, without any biological experimental evidence, might be responsible for the translocation of RNA and protein chains through small nuclear pores in eukaryotic cells. On the basis of our previous studies of the breathing motions of porous hollow spheres in liquid and the translocation of polymer chains with different topologies through a small cylindrical pore, we conjecture that a thermally agitated vibration (breathing motion) of the nuclear membrane passively generates a flow through each nuclear pore (a velocity gradient at its entrance) sufficiently higher than the critical (minimum) flow rate for linear chains to pass through a small pore, irrespective of their lengths, such that linear protein and

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RNA chains with different lengths can be naturally driven into and out of the nucleus without any collision. It should be stated that our hypothesis could not explain why in prokaryotic cells RNA and protein chains are also linear. Obviously, the genetic coding and biochemistry must play a very important and determined role here. Our hypothesis might have some implication in the evolution and formation of the nuclear pores and membrane.

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Notes

The authors declare no competing financial interest.

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