

Within the channel, cargos move in a non-directional manner, consistent with anomalous subdiffusion in a crowded volume, with dimensions of ~55 nm in width and ~68 nm in length. By varying the number of import receptors on the surface of the cargo, we find that the translocation is not governed by simple receptor-NPC binding interactions and that the central channel behaves in accordance with the 'selective phase' model. Finally, in the absence of Ran, cargos still explore the entire volume of the NPC, but have a dramatically reduced probability of exit into the nucleus from the pore, suggesting that NPC entry and exit steps are not equivalent and that the pore is functionally asymmetric to importing cargos.

1603-Pos

Single Molecule Imaging of the Calcium Ion Regulation of Nuclear Pore Passive Permeability

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Nuclear pore complex (NPC) is the sole pathway for direct communication between the cytoplasm and the nucleoplasm of eukaryotic cell. Based on the criteria of the molecular size exclusion, the NPC allows unregulated passive diffusion of small molecules (< 40 kDa) and facilitated translocation of larger molecules (up to 50 MDa). While recent evidence suggests a third transport mode: Ca^{2+} regulated transport. In details, the nuclear pore permeability can be regulated by Ca^{2+} stored in the lumen of nuclear envelope and endoplasmic reticulum. However, the mechanism of Ca^{2+} regulated transport remains poorly understood. Here we applied a speedy single molecule fluorescence microscopy to characterize the dependence of the nuclear pore passive permeability on the Ca^{2+} store concentration by snapshots of real-time transient movements of 3 - 40 kDa dextran molecules through the NPCs. We observed novel features under real-time trafficking conditions that escape detection by ensemble measurements: decreased amount of Ca^{2+} in the store induced restricted passive diffusion of dextran molecules with longer diffusion times and lower transport efficiencies through the NPCs. Dextran molecules cannot penetrate deeply into the nuclear pore and the majority were rejected or trapped by likely barriers formed on both sides of NPCs when the stored calcium was significantly depleted. Our results suggest that a filamentous structure that occludes the NPC may be altered by the depletion of calcium in the store.

1604-Pos

Single-Molecule Snapshots of Three-Dimensional Distribution of Transient Interactions in the Nuclear Pore Complex

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Translocation of macromolecules through the nuclear pore complex (NPC) is hindered by the phenylalanine-glycine (FG) repeats barrier unless they are chaperoned by transport receptors in eukaryotic cells. However, challenged by measuring a series of transient interactions between the transport receptor and the FG repeats, the precise mechanism of the facilitated translocation remains unclear. We applied single-point edge-excitation sub-diffraction (SPEED) microscopy to obtain a three-dimensional density map of the transient interactions with a spatiotemporal resolution of 9 nm and 400 μs . We observed novel features under real-time trafficking conditions that escape detection by conventional electron microscopy: the actual pathway of facilitated translocation through the NPC is not completely restricted by the NPC architecture; the primary interaction sites between Importin $\beta 1$ (Imp $\beta 1$, a major transport receptor) and the FG repeats locate symmetrically on the cytoplasmic and nucleoplasmic sides of the nuclear pore; Imp $\beta 1$ only rarely occupies a central channel of approximate 10-20 nm diameter along the NPC axis, but the Imp $\beta 1$ -assisted cargo molecules expand their pathways into the central channel.

1605-Pos

Squeezing through the Pore - Conformational Plasticity in Nuclear Import

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In eukaryotic cells, exchange of macromolecules between the cytoplasm and the nucleus is mediated by specialized transport factors. By binding to these transporters, cargo molecules which are otherwise excluded from entering the nucleus can traverse the nuclear pore efficiently. Most of the proteins mediating nuclear import and export belong to the importin beta family. The transport cycle of importin beta starts with recognition of the cargo in the cytoplasm. The importin-cargo complex then crosses the permeability barrier of the nuclear pore and enters the nucleus, where the complex dissociates upon binding of RanGTP to importin beta.

Importin beta has a superhelical structure and exhibits a great intrinsic flexibility, which is needed for recognition of a wide variety of ligands [1]. Although small angle scattering data, as well as previous molecular dynamics studies, in-

dicate that in solution importin beta adopts a rather open conformation, most crystal structures exhibit a compact conformation of the superhelix [2, 3].

We carried out all-atom molecular dynamics simulations of importin beta in aqueous solution and in hydrophobic media and found a large influence of the hydrophobicity of the environment on conformation and dynamical properties of importin beta. Our results indicate that the structural plasticity of importin beta is not only important for cargo recognition, but that its dependence on the environment plays an important role in the transport process. We propose a mechanism by which opening and closing of importin beta during nuclear import facilitate cargo binding, transport and release.

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[2] Fukuhara, N.; Fernandez, E.; Ebert, J.; Conti, E. & Svergun, D. *J Biol Chem* 2004, 279, 2176-2181.

[3] Zachariae, U. & Grubmüller, H. *Structure* 2008, 16, 906-915.

1606-Pos

Polymer Brushes and the Nuclear Pore Complex

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The nuclear pore complex (NPC) is an important macromolecular structure that gates the aqueous pores between the cytoplasm and nucleoplasm of cells and controls all nucleo-cytoplasmic transport and communication such as the import of proteins from the cytoplasm and the export of RNA from the nucleus. The NPC forms a barrier that maintains a tight seal against cytoplasmic particles larger than 4 nm while simultaneously allowing the facilitated transport of specially "tagged" particles up to 40 nm diameter, at speeds comparable to free diffusion! The key to the selectivity is hypothesized to be due to a large number of NPC proteins that fill the pore and potentially interact with each other and the cargo. However, despite numerous studies on the structure and properties of individual NPC proteins, the actual structure of the complex within the nuclear pore and its mechanism of operation are virtually unknown with leading models of nuclear pore transport assuming vastly different morphologies for the NPC protein complex filling the nuclear pore. Here, we use a bottom-up approach, applying the physics of polymer brushes to understand the three dimensional architecture of the complex based on experimental understanding of the properties of individual NPC proteins. Our results indicate that there exist transitions between distinct brush morphologies (open and closed states of the gate), which can be triggered by the presence of cargo with specific surface properties. This has led to development of the Discrete Gate Model - an experimental data driven theoretical model. The resulting transport mechanism, that we propose, is fundamentally different from existing models and points to a novel form of gated transport in operation within the nuclear pore complex. Our results can also be extended to designing and optimizing novel forms of biomimetic transport based on this mechanism.

1607-Pos

Formation of the Bicoid Gradient in *D. melanogaster* in Unfertilized Versus Fertilized Eggs

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The concentration profile of Bicoid protein in *D. melanogaster* is an example of a morphogen gradient that has been well studied by quantitative methods. Various models have been proposed which suggest that the layer of syncytial nuclei which forms in the embryo prior to gastrulation plays a significant role in the formation of this gradient. In particular, given the small cytoplasmic diffusion constant of 0.3 microns²/s reported by Gregor *et al.*, it has been proposed that the nuclear motion coincident with mitotic divisions serves to extend the length scale of the gradient. In this work we present time series measurements of the Bicoid gradient in unfertilized eggs, which lack nuclei other than a single female pronucleus. We find that the unfertilized gradient takes a form similar to the fertilized gradient modulated by a uniform positive offset across the egg, consistent with the claim that syncytial nuclei do not extend the length of the gradient. In addition, we present results of simulation to argue that nuclear trapping combined with observed nuclear motion is insufficient to resolve the paradox of a small diffusion constant and an embryo-length gradient.

1608-Pos

Endothelin and Phenylephrine Both Trigger Nuclear IP₃ Elevation, but Differ in Ability to Activate Nuclear HDAC5 Export

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