Nucleocytoplasmic Transport

1363-Pos Board B273
Single Molecule Studies of Nuclear Transport
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The translocation of single biomolecules through nanopores is one of the most fundamental processes of life. For example, the import of histones into the cell nucleus via the nuclear pore complex (NPC) is crucial for DNA condensation. We aim to gain a deeper understanding of NPC translocation on a single molecule level by combining optical tweezers with patch clamp techniques. This gives us the unique possibility to analyze the interaction forces between a biological macromolecule and the NPC while measuring the relevant time scales by electrophysiological recordings.

We present preliminary results on the characterization of the properties of NPCs concerning mode and duration of macromolecular translocation. Nuclei extracted from the syntactical coconut coccus nucifera are one model system. We demonstrate the selectivity of the NPC using an import signal tagged with GFP. We determine the conductance of a single nuclear pore to be approximately 490 pS. Our results allow for estimation of the nuclear pore density to be between one and four per micrometer. From observations of the ionic current across the pores, we are able to observe gating and to derive the translocation time of cargo across single NPCs.

As a technical development towards nuclear pore force spectroscopy we introduce optical fiber illumination for real-time tracking of optically trapped particles. With this approach, we are able to observe the interaction forces between single molecules and the NPCs under real-time trafficking conditions. Using a deconvolution algorithm, we advanced the single-molecule tracking (SPT) approach to follow a single NPC. While we track the pore, single molecules pass through it, and their interactions with the NPC can be spatially and functionally regulated by transport receptors. Our data strongly support a transport mechanism in which transport receptors or transport-receptor-cargo complexes pave the pathways by interacting with the NPC.

References

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Single-Molecule Studies of Nucleocytoplasmic Transport: from 1D to 3D
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The nuclear pore complex (NPC), the sole gateway for nucleocytoplasmic exchange in eukaryotic cells, allows for the passive diffusion of small molecules (<20-40 kDa) and the transport-receptor-facilitated translocation of larger molecules. However, the precise transport mechanism as to how these two transport modes pave their pathways through the NPC remains in dispute among numerous transport models. By a newly developed single-molecule speed microscopy and a deconvolution algorithm, we advanced the single-molecule imaging of nucleocytoplasmic transport from 1D (one-dimension) to 3D. The 3D routes for both passive and facilitated transport through human NPCs under real-time trafficking conditions have been obtained with a spatial resolution of 9 nm at 400 μs. Our data strongly support a transport mechanism in which transport receptors or transport-receptor-cargo complexes pave their pathways by interacting with the FG repeats at the periphery around a single primary central axial channel for passive diffusion of small molecules.

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Three Dimensional Dynamic Nuclear Transport Pathways Illuminated by Single-Molecule Microscopy
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The nuclear pore complex (NPC), the sole gateway for nucleocytoplasmic exchange in eukaryotic cells, allows for the passive diffusion of small molecules and the transport-receptor-facilitated translocation of larger molecules. However, whether these two transport modes spatially separate or joined pathways and whether they functionally conduct uncoupled or coupled transduction remains in dispute. By the single-molecule snapshots of transient diffusion or interactions through native NPCs, we have determined the three-dimensional transport pathway for each mode is mainly separate. Both pathways can be spatially and functionally regulated by transport receptors. Our data strongly support a transport mechanism in which transport receptors or cargo complexes pave the pathways by interacting with the phenylalanine-glycine repeats at the periphery around a single primary central axial channel for passive diffusion.

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Nuclear Export of mRNA in Living Cells
David Grünwald.

An important step in the life cycle of mRNA is its export from the nucleus to the cytoplasm, which is mediated by nuclear pores, large nano-machines with diameters of roughly 120 nm. As a result, mRNA export occurs over distances smaller than the optical resolution. While there is extensive knowledge of the physical structure and composition of the NPC transport selectivity and dynamics of mRNA export at nuclear pores remain unknown. Major models for nuclear pore function are currently focusing on the central channel of the pore, but we spatially resolved the kinetics of mRNA transport and present a three step model consisting of docking (80 ms), transport (5-20 ms) and release (80 ms), totalling 180 ± 10 ms. Here, access and release from the pore complex are rate-limiting steps with the channel translocation time being in the order of transport dwell times found for protein import to the nucleus.

We developed a super-registration approach using fluorescence microscopy overcoming the current limitations of colorization by means of measuring intermolecular distances of chromatically different fluorescent molecules with nm precision. With this method we achieve 20 ms time- and at least 26 nm spatial precision, rendering the capture of highly transient interactions in living cells possible.

1367-Pos Board B277
Tracking Single Nuclear Pore Complexes in Live Cells: Importin β Transport and Beyond
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The molecular details of transport through nuclear pore complexes (NPCs) have been well characterized, including the key role of Importin Beta (ImpB) receptor. However, the overall system behavior in intact cells is difficult to analyze because of its highly dynamic nature. Contrary to the common single particle tracking (SPT) approach that tracks an isolated particle as it moves through the pore, we set out to track the center of mass of the entire single NPC. While we track the pore, single molecules pass through it, and their location and dynamics are analyzed by fluorescence correlation spectroscopy (FCS). By this unconventional approach we find that ImpB transport is regulated so as to produce a characteristic bump in the autocorrelation function at the NPC. This regulation is spatially restricted to the pore, dependent on ImpB properties, pore structure, and metabolic energy. Combined to simulations our results suggest that ImpB movement within the pore is likely to be directed instead of unbiased, and that the back and forth components of its shuttling are coupled in time. We use our results to discriminate between existing NPC functional models, and identify key features that must be essential for transport in the intact pore.

Intracellular Channels/IP3 Receptors

1368-Pos Board B278
TRIC-A Channel and Blood Pressure Regulation
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Trimeric intracellular cation (TRIC) channel subtypes, namely TRIC-A and TRIC-B, function as intracellular channels conducting monovalent cations throughout tissues and probably mediate counter-ion movements coupled with Ca2+ release from the endo/sarcoplasmic reticulum. Knockout mice lacking both TRIC-A and TRIC-B channels suffer embryonic cardiac failure, and the mutant cardiomyocytes display severe dysfunction in SR Ca2+ handling (Yazawa et al., Nature, 2007). In knockout mice lacking TRIC-B channels with neonatal lethality due to respiratory failure, alveolar epithelial cells exhibit compromised Ca2+ release and thus insufficiently produce and secret surfactant phospholipids (Yamazaki et al., Development, 2009). The observations indicate that TRIC channels act as counter-ion channels functionally coupled with Ca2+ release in various cell types.

Here we report the direct linkage of TRIC channels with hypertension. Knockout mice lacking TRIC-A channels showed significant hypertension and bradycardia. Ca2+ channel antagonists, but not blockers for vasoactive agents, exerted antihypertensive effects in the mutant mice. Moreover, despite retaining normal passive diameters in a Ca2+-free bathing solution, the mutant arteries showed narrow diameter results from higher resting Ca2+ level maintained by Ca2+ influx via L-type Ca2+ channel. In vascular smooth muscle cells (VSMCs), spontaneous opening of ryanodine receptor channels generates local Ca2+ release called Ca2+ sparks, activates cell-surface Ca2+-dependent K+ channels and reduces membrane potential toward relaxation. Our physiological and pharmacological studies suggested that the loss of TRIC-A channels.

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