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 β1 (Imp β1, a major transport receptor) and the FG repeats locate symmetrically on the cytoplasmic and nucleoplasmic sides of the nuclear pore; Imp β1 only rarely occupies a central channel of approximate 10-20 nm diameter along the NPC axis, but the Imp β1-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, indicate 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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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 syncitial 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 microns^2/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 syncitial 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 IP_3 Elevation, but Differ in Ability to Activate Nuclear HDAC5 Export

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We previously found that both ET-1 and phenylephrine (PE) induced similar HDAC5 phosphorylation and nuclear export in adult cardiac myocytes (which contributes to hypertrophic signaling). However, ET-1 requires IP_3 receptor (IP_3R) activation (at the nucleus) and CaM-CaMKII activation to mediate this full effect, while PE does not. That is, IP_3R inhibition or CaMKII block do not prevent PE-induced HDAC5 nuclear export, despite the fact that both agonists can activate IP_3 production. Here we test whether the apparent IP_3-independence of PE signaling is due to a failure of IP_3 elevation in the nucleus (i.e. IP_3 produced at the plasma membrane may be degraded before reaching the nucleus). Using a nuclear targeted FRET-based IP_3 sensor (Fire-1-Nuc) we assessed changes in nuclear [IP_3] upon ET-1 and PE application in adult rabbit ventricular myocytes. Both ET-1 and PE induce rapid and robust elevation of nuclear [IP_3] reaching an early peak in <1 min. While the ET-1-induced a slightly larger peak nuclear [IP_3], the PE-induced rise is more sustained (lasting more than 10 min). These results demonstrate that a PE induces a strong rise in nuclear [IP_3], and does not support the hypothesis that PE fails to induce a nuclear IP_3 signal (compared to ET-1). We cannot rule out the possibility that the kinetic differences in nuclear [IP_3] between these agonists contribute to different downstream signaling. Another explanation is that PE-induced nuclear IP_3 is less effective than ET-1-induced IP_3 in driving activation of nuclear CaM and CaMKII to phosphorylate HDAC5. That is, an IP_3-independent effect of one of these agonists could prevent or promote the ability of IP_3 to signal in the nucleus.

1609-Pos

Phosphorylation Dependent Nuclear Transport of Human Dutpase

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The nuclear isoform of human dUTPase plays an important role in maintaining genomic integrity. Its expression is strictly cell cycle regulated and is known to be a phosphoprotein in vivo. However, the role of this phosphorylation remained unknown.

Here we show regulation of the nuclear transport of human dUTPase via phosphorylation of a serine residue on its nuclear localisation signal. We found that hyperphosphorylation mimicking mutants (glutamic acid) are localized solely in the cytoplasm while hypophosphorylation mimicking mutants (glutamine) localize in the nucleus as the endogenously regulated protein. Our video microscopy studies have also shed light on the nuclear import dynamics of the wild type dUTPase and that of the mutants. These results showed that the phosphorylated wild type form may re-enter the nucleus (after cell division) only after a considerable delay of several hours while mutants that cannot be phosphorylated re-accumulate within the nucleus much faster. The delay observed with the wild type enzyme may indicate that either dephosphorylation or de novo protein synthesis is required. To reveal the mechanism by which cells accumulate sufficient amount of dUTPase in their nucleus after cell division, we are currently conducting protein transfection based experiments.

We are also trying to characterize the interaction of the human dUTPase with its possible partner in nuclear trafficking, importin-alpha. Based on Native-PAGE and ThermoFluor experiments, we detected a relatively high affinity complex of dUTPase with importin-alpha. Complex formation was also observed in the case of the hypophosphorylation mimicking mutant (S11Q), but not with the hyperphosporylation mimicking mutant (S11E). We also conduct crystallographic studies of the complex using various dUTPase NLS peptides.

Voltage-gated Na Channels II

1610-Pos

Stable Expression of Brain Sodium Channels in Human Cells by Multiplexed Transposon-Mediated Gene Transfer

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Generation of cultured human cells stably expressing one or more recombinant gene sequences is a widely used approach in biomedical research, biotechnology and drug development. Conventional methods are not efficient and have severe limitations especially when engineering cells to co-express multiple transgenes or multi-protein complexes. We harnessed the highly efficient, non-viral and plasmid-based *piggyBac* transposon system to enable concurrent genomic integration of multiple independent transposons harboring distinct protein-coding DNA sequences. Flow cytometry of cell clones derived from a single multiplexed transfection demonstrated ~60% (three transposons) or ~30% (four transposons) co-expression of all delivered transgenes despite selection of a single marker transposon. We validated multiplexed *piggyBac* transposon delivery by co-expressing large transgenes encoding a multi-subunit neuronal voltage-gated sodium channel (SCN1A) containing a pore-forming subunit and two accessory subunits while using two additional genes for selection. Previously unobtainable robust sodium current was demonstrated through 38 passages, suitable for use on an automated high-throughput electrophysiology platform. Co-transfection of three large (up to 10.8 kb) *piggyBac* transposons generated a heterozygous SCN1A stable cell line expressing two separate alleles of the pore-forming subunit and two accessory subunits (total of four sodium channel subunits) with robust functional expression. We concluded that the *piggyBac* transposon system can be used to perform multiplexed stable gene transfer in cultured human cells and this technology may be valuable for applications requiring concurrent expression of multi-protein complexes.

1611-Pos

The Functional Effect of R1648H, a Sodium Channel Mutation that Causes Generalized Epilepsy with Febrile Seizures Plus in Splice Variants of SCN1A

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SCN1A, the gene that encodes the alpha subunit of the voltage-gated sodium channel Nav1.1, is alternatively spliced at exon 5. SCN1A contains two copies of exon 5, denoted 5N and 5A (for 'Neonatal' and 'Adult' according to their developmental expression). There are 3 amino acid substitutions between the splice variants, all within the D1:S3/S4 extracellular linker. It is unknown how exons 5N and 5A alter channel function. Because patients with Generalized Epilepsy with Febrile Seizures plus (GEFS+) frequently exhibit age-dependent changes in seizure frequency and severity, we have asked whether the GEFS+-associated SCN1A mutation R1648H differentially affects Nav1.1-5N and Nav1.1-5A.

We examined brain tissue obtained from patients undergoing epilepsy surgery to examine the relative proportion of SCN1A transcripts containing exons 5A and 5N. A significantly greater proportion of Nav1.1 mRNA in epilepsy tissue contain exon 5N than in control brain tissue. We expressed either splice variant of SCN1A in HEK293 cells, and recorded whole-cell currents with a CsCl-based pipette solution. Nav1.1-5N demonstrated a leftward shift of both activation (Nav1.1-5N: V50= $-18.3 \pm$-0.6 mV ; Nav1.1-5A: $-15.3 \pm$-0.5 mV; $P<0.05$) and inactivation (Nav1.1-5N: V50= $-60.0 \pm$-1.0 mV; Nav1.1-5A: $-54.0 \pm$-1.1 mV). The GEFS+ mutation R1648H, did not affect activation or current density for either variant. The mutation also failed to increase the size of the persistent current evoked by prolonged depolarising steps. Instead, a hyperpolarizing shift in inactivation was observed when the mutation was expressed in Nav1.1-5A but not Nav1.1-5N channels (mutant: V50 inactivation = $-60.9 \pm$-1.0 mV; wild-type: $-54 \pm$-1.1 mV). This suggests that R1648H leads to a net loss of function in adult neurons. This effect may lead to an impairment of recruitment of GABAergic interneurons that preferentially express Nav1.1.

1612-Pos

Traumatic Brain Injury and Axonal Sodium Loading: Modeling the Impact of Left-Shifted Nav Channel Operation at Blebbed Nodes of Ranvier

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Traumatic brain injury like stretch immediately (<2 min) and irreversibly causes a TTX-sensitive axonal [Ca2+] increase that, in situ, underlies an untreatable pathology, diffuse axonal injury. Nav1.6-expressing mammalian cells, we showed, immediately (<2 min) exhibit TTX-sensitive Na+-leak following traumatic stretch (Wang et al 2009 Am J Physiol 297: in press). In situ, even mild axonal stretch injury can trigger adverse positive feedback so that leaks progress irreversibly to lethality. Though clinical trials are underway using Nav channel blockers that might reduce the severity of this outcome, molecular understanding of Nav channel damage has been lacking. Recently, however, we showed that activation and steady-state inactivation of recombinant Nav1.6 channels both irreversibly left-shift (up to -20 mV) in traumatized membrane (Wang et al 2009) as if their voltage sensors are responding to the increased bilayer disorder of traumatized (blebbed) membrane. In axonal membrane traumatized to various extents, this should smear out the window current range leftward between the normal range toward the resting potential range,