and tubulin surface, two of which are involved in stabilizing the extra turns of switch II helix (α4) formed toward the nucleotide-binding pocket. In contrast, only few salt bridge formations are possible in ADP state, explaining why ADP release causes specific and tight binding to microtubule. The structural change of α4 promotes hydrogen bond and hydrophobic interactions of highly conserved residues in α4 with switch II loop, pulling switch II loop away and promoting ADP release from nucleotide pocket. ADP release and ATP binding cause rotational movements of α4 and also rotational movements of nucleotide-binding P-loop and its surrounding elements. These nucleotide-dependent domain motions alter the mobility of the neck linker, providing structural basis for how kinesin's two motor domains coordinate to move processively.

1923-Pos

Probing the Mechanism of Kinesin-1 Motion in Three Dimensions Using the Photonic Force Microscope

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Kinesin-1 is a molecular motor essential for cellular function. It transports components around the cell by a processive movement along microtubules while hydrolysing ATP. Although extensively studied by a variety of techniques, the mechanism used by these single-molecule motors to produce this efficient motion on the nanometer scale is not fully understood.

In our investigations we use the Photonic Force Microscope (PFM) to trap and track a 500nm bead attached to a kinesin motor as it interacts with a microtubule *in vitro.* The PFM is an optical trap capable of recording a trapped dielectric particle's motion in three dimensions with nanometre spatial and microsecond temporal resolutions. Using the data recorded we can infer information about the molecular motor's position and its mechanical properties. By characterising different conformational states of the kinesin molecule from its changing mechanical properties as it processes, we expect to learn more about the cycle of events that make kinesin movement possible.

An understanding of how nature achieves this motion on the nanoscale will help combat diseases related to kinesin's malfunction and will allow production of similar artificial nanomachines in the future.

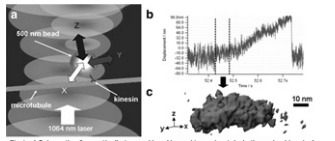

Fig.1: a) Schematic of an optically trapped bead bound to a microtubule through a kinesin-1 molecule. b) Position trace of a bead attached to a kinesin motor travelling along a microtubule orientated in the Y-axis. c) 3D iso-energy profile of bead centre positions in the bound state highlighted on graph.

1924-Pos

Multiple Interacting Kinesin-1 Motors Cooperate Negatively

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Many sub-cellular commodities are transported by more than one motor, and it is well-known that the combined function of motors can lead to unique transport behaviors. Yet, little is known about how grouping multiple motor proteins influences the motile properties of cargos, and in particular, relationships between the structural / compositional organization of motor complexes and key collective transport parameters (run lengths, detachment forces) have not been established.We have taken important steps towards solving this problem by synthesizing the first set of structurally-defined complexes of interacting kinesin-1 motors. Furthermore, we have developed 'single-molecule' assays that can examine new and important aspects of collective motor dynamics; namely, whether multiple motors cooperate in a positive or negative fashion and if these behaviors influence ensemble transport properties of multiple motor systems. Herein, we demonstrate that interactions among two elastically-coupled kinesin molecules lead to negative motor cooperativity, and that this behavior influences collective motor force production. We also describe how such effects can reconcile differences between measurements of cargo motions *in vitro* and in living cells.

1925-Pos

Kinesin-1's Behavior at Obstacles

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Using single molecule stepping assays, we were able to show that kinesin-1 stops when it encounters an obstacle in its path on the microtubule lattice. Based on the stepping mechanism of kinesin-1, we propose the following model to explain why the molecule stops at obstacles:

Kinesin-1's processivity requires the rear head to stay bound until the leading head is firmly attached to the next tubulin dimer. The fact that kinesin-1 follows a single protofilament limits the choice of forward binding to the next tubulin dimer along the same protofilament. Therefore, if a large molecule is blocking the next tubulin dimer, the leading head cannot bind and the rear head cannot detach. This situation effectively stalls the kinesin-1 molecule until it detaches from the microtubule or a forward binding site becomes free.

Based on this model, we were able to calculate the dissociation rate of kinesin-1 in the stopped state. This calculated value agreed very well with a direct measurement, indicating that the model accurately describes kinesin-1's behavior at obstacles. A very similar dissociation rate has been measured previously for single-headed kinesin-1 mutants, suggesting that kinesin-1 waits at obstacles in a one-head bound state.

Interestingly, in about 50 % of the observed stopping events, kinesin-1 did not detach at the end of the stopping phase, but overcame the obstacle and continued to walk. The rate with which kinesin-1 exited the stopped phase by overcoming the obstacle was almost identical to the dissociation rate measured for stopping events. Therefore, it is likely that kinesin-1 overcomes a roadblock by detaching from the microtubule and then, instead of leaving into solution, reattaching next to, or behind the obstacle.

1926-Pos

***In Vitro* Analysis of the Effect of Microtubule Acetylation on Kinesin Motility**

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Plus end-directed intracellular transport by kinesins on microtubules in eukaryotic cells directs cargo to the cell's periphery, but to carry out polarized transport, additional signals from microtubules must be recognized by cargo-carrying kinesins. One emerging hypothesis, supported by *in vivo* observations of preferential kinesin-1 transport along acetylated microtubules, suggests that post-translational modifications (PTMs) of tubulin subunits in subsets of microtubules serve as markers for intracellular transport. Here we are examining if and how acetylation of microtubules directly regulates kinesin motility. As a source of acetylated and unacetylated microtubules, we have used *Tetrahymena* doublets extracted from a wild type strain and a mutant strain wherein the otherwise acetylated Lysine-40 is mutated to an Arginine. For obtaining fluorescently-labeled kinesin, lysates were extracted from COS cells transfected with Kinesin-1 genetically labeled with three-tandem monomeric citrines (3xmCit-KHC). To evaluate the effect of acetylation on Kinesin-1 motility, we used TIRF (Total Internal Reflection Fluorescence) microscopy to perform single molecule *in vitro* motility assays and measure the velocity and run length of 3xmCit-KHC on acetylated and unacetylated doublet microtubules. Our observations show that while the *in vitro* velocity remains unaltered, twice as many binding events can be observed for 3xmCit-KHC on wild-type doublets than on unacetylated doublets. We conclude that the motor domain of Kinesin-1 directly recognizes acetylation of microtubules and has a greater tendency to bind acetylated microtubules than unacetylated microtubules. We suggest that acetylation of microtubules enhances the binding affinity of Kinesin-1, which in turn allows preferential transport by Kinesin-1 along acetylated microtubules. To exclude differences between motility assays as source for the observed preferential binding of Kinesin-1 to acetylated microtubules, we are now comparing the binding and motility of Kinesin-1 for acetylated and unacetylated microtubules in the same motility assay.

1927-Pos

Surface Passivation for Molecular Motor Protein Assays

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In kinesin motiliy assays, it has been shown that the surfaces with which kinesin interacts must be passivated in order to prevent kinesin from denaturing on them. The most popular surface blocker is the casein family of milk proteins. Casein is usually purified to various degrees from bovine milk and has many unknowns associated with it when reconstituted and used in motor protein assays. In order to obtain a clearer picture of how kinesin and microtubules interact, a cleaner surface passivation needs to be found. The interaction of kinesin with microtubules has been studied extensively, however, there are fewer studies that investigate how the interaction of kinesin and microtubules changes due to surface passivation. One recent study has shown that the differing components of casein (termed alpha, beta, and kappa) can significantly affect microtubules in gliding motility assays [1]. Gliding motility assays are assays where a glass cover slip is passivated and kinesin is prevented from interacting directly with the substrate. Microtubules are then propelled by the motor activity of a bed of immobilized kinesin molecules. Lipid molecules are fatty acids that can be purified to a much greater extent than casein can. Also, lipid molecules exhibit the same amphiphilic behavior as casein, they adhere to glass easily, and can be easily functionalized. Lipids are thus an attractive alternative

to casein proteins for surface passivation. We report preliminary results on the surface passivation performance of lipid molecules and other materials in gliding motility assays. [1] Vivek Verma, William O Hancock, Jeffrey M Catchmark, ''The role of casein in supporting the operation of surface bound kinesin,'' J. Biol. Eng. 2008; 2: 14. PMID: 18937863

1928-Pos

"Popoffs" Under a Transverse Force Reveal the Number and Location of Active Kinesin Motors During Motility Assays

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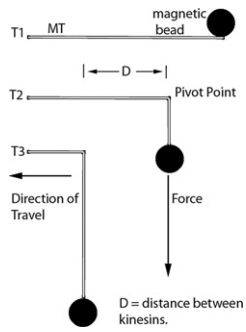
Determination of the number of active motors pulling a single MT or bead during motility assays has proven difficult. Traditional protein concentration assays, such as Bradford, cannot distinguish between active and inactive motors. We attach a superparamagnetic bead to the (+) end of a microtubule. When placed in a magnet with uniform magnetic field gradient, the bead pulls on the MT with a controllable 0-10 pN force. If the force is perpendicular to the gliding direction of the MT, a short section of the MT ''pops off'' the surface every 2 to 5 s, as shown in the diagram. This detachment is characterized by rapid motion of the superparamagnetic bead in the direction of higher magnetic field gradient followed by normal microtubule gliding velocity when the MT is pulled taut. The length of the short section between ''popoffs'' is the distance between active kinesins along a microtubule.



1929-Pos

Calcium Dependent Regulation of Kinesin Function using Binding System of CaM and M13 Peptide

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Kinesin is known as a dimeric motor protein, which carries cellular cargoes along microtubules by hydrolyzing ATP. Calmodulin (CaM) is a calcium binding protein that participates in cellular regulatory processes. CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. We have previously demonstrated that kinesin fused with CaM at the C-terminal binds reversibly to M13-Qdots in a calcium dependent manner. In this study, we have tried to make the calcium dependent reversible dimerization of kinesin utilizing CaM- target peptide M13 binding system in order to control motility of kinesin. First we have designed and prepared the cDNA of the truncated kinesin (355 amino acids) that does not form dimer. Subsequently we prepared the cDNA encoding two kinesin chimeric proteins in which C-terminal of kinesin355 was fused with calmodulin (kinesin355-CaM) and fused with M13-GFP (Kinesin355-M13-GFP). The cDNAs of the kinesin chimeras were cloned into expression vector pET21a and transformed into E.coli BL21. The kinesin chimeras were successfully expressed and purified by Co-Chelate column. These kinesin chimeras showed normal ATPase activities. Furthermore, K355-CaM bound to M13-YFP in a calcium dependent manner. And the calcium dependent interaction between kinesin355-CaM and kinesin355-M13-GFP was examined.

Membrane Transport

1930-Pos

The Sodium-Glucose Co-Transporter SGLT1 - Could Light Help Prevent Type II Diabetes?

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The sodium-glucose co-transporter SGLT1 is responsible for the active transport of glucose in small intestine and kidney. Consuming food with high degrees of carbohydrates and glucose leads to a temporary, rapid increase of blood glucose levels via the absorption of glucose and galactose through SGLT1. This influences the glucose homeostasis and increases the insulin resistance of peripheral tissue. The subsequent ''glucose-toxicity'' leads to degeneration of beta-cells and, in last consequence, to the generation of type II diabetes. The reduction of oral glucose availability by inhibition of SGLT1 with flavonoids or other ''nutraceuticals'' might be one possibility to prevent type II diabetes.

The transport of glucose via SGLT1 is electrogenic and coupled to the co-transport of sodium ions. Its features are examined using cell-free, solid-supported-membrane-based electrophysiology, namely the SURFE^{2}R technology platform (IonGate Biosciences), where transporter-containing membrane fragments or vesicles are mechanically and electrically coupled to a gold-coated biochip. For SGLT1, it is important to establish a membrane potential prior to substrate application, to enhance the sensitivity of the assay. This potential can be built up via a SO_4/Cl^- gradient across the membrane. The following detectable transport activity is in the range of 300-1000 pA.

To avoid unspecific side effects and to speed up screening, the electrochemical SO_4/Cl^- gradient can be replaced by a light-driven gradient. Therefore, we generated cell lines with light polarizable membranes, where the application of light generates a membrane potential. With this technique it is possible to achieve higher throughput and a better signal-to-noise ratio in drug screening.

1931-Pos

Adaptation of Animals to Different Types of Oxidative Stress: The Role of Mitochondrial Potassium Transport Systems

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We studied parameters of the ATP-dependent influx of potassium into mitochondria, which were isolated from rats varying in their resistance to ischemia and from hypoxia-adapted animals. It has been found that in the heart and liver mitochondria, the rates of the ATP-dependent potassium influx and H_2O_2 production (in case of ATP-inhibited transport) are higher in the hypoxia-resistant rats, as compared to those in the hypoxia-sensitive animals. When adapted to low oxygen, the hypoxia-sensitive rats demonstrated rates of the both processes increasing to the levels observed in the hypoxia-resistant animals. However, the concentration of potassium in the mitochondria of hypoxia-resistant and adapted animals decreased. This indicates that adaptation to hypoxia stimulates not only the influx of potassium into mitochondria, but also K^+/H^+ exchange. The activation of such a potassium cycle can lower the production of ROS, which plays a crucial role in the lethal cell injury associated with cardiac ischemia and reperfusion. It has been further found that uridine and UMP (precursors of UDP, a metabolic activator of mitoK_{ATP}) greatly decreased the index of ischemic alteration upon 60-min acute ischemia, as well as the size of infarction zone under ischemia-reperfusion conditions. The inhibitors of K_{ATP} channels (glibenclamide and 5-HD) reversed the anti-ischemic effect of uridine and UMP. These agents also exerted an anti-arrhythmic effect, which was completely abolished by glibenclamide but not 5-HD. It should be noted that uridine and UMP recovered the levels of ATP, phosphocreatin and glycogen, which were decreased during ischemia, while glibenclamide and 5-HD eliminated these effects. Also demonstrated was the effectiveness of uridine in the reduction of lipopolysaccharide-induced inflammation (another model of oxidative stress).

1932-Pos

Investigation of Proton-Potassium Exchange During Fermentation of Glycerol by Bacteria *escherichia Coli* at Alkaline and Acidic pH

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Production of molecular hydrogen (H_2) by bacteria from a variety of renewable, cheap and abundant carbon sources is a developing new area of technology. Recently it has been shown that bacteria *Escherichia coli* is able to ferment glycerol and produce H_2 via formate hydrogen lyase (FHL) system probably (1). It was demonstrated that in *E. coli* during fermentation of glucose depending of medium pH H_2 produces via two forms of FHL-1 and FHL-2, constituted by formate dehydrogenase H and hydrogenase 3 (H3) or hydrogenase 4 (H4): at alkaline pH FHL-2 was shown to relate with the proton translocating F_0F_1-ATPase and potassium uptake TrkA system (2).

In this study it's shown that at acidic (pH 5.5) and alkaline (pH 7.5) medium in *E. coli* wild typefermenting glycerol protons expelled via F_0F_1-ATPase with low rate compared with the glucose fermentation. The potassium uptake was very low. During fermentation of glycerol at alkaline pH H^+ extrusion was stimulated in *ΔhyfG or ΔfhlA* (with defective H4 or activator of FHL, respectively)and not markedly changed in *ΔhyaB orΔhybC* mutants (with defective H1 or H2). The H^+ extrusion was almost the same in all these mutants at acidic pH. The results indicate that at alkaline pH when F_0F_1-ATPase activity is low H3 or H4 but not other hydrogenases may participate in the H^+ extrusion or have proton translocating ability.