chemosensory cilia of Caenorhabditis elegans two kinesin-2-family motors, kinesin-II and OSM-3-kinesin, cooperate to build and maintain the cilium, in a process called intraflagellar transport (IFT). In order to quantitatively asses IFT-kinesin function at endogenous expression levels, we have generated transgenic worms using Mos1-mediated single-copy integration of transgenes encoding fluorescently-labeled-IFT kinesins. Ultrasensitive wide-field and confocal fluorescence microscopy allows accurate mapping, counting, tracking and correlation of these molecular machines inside living, multicellular organisms. This approach allows unprecedented insight into IFT and motor-driven processes in general.

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Coarse-Grained Model of Cooperative Chloroplast Transport in Moss

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Organelle motility is essential for the functioning of the eukaryotic cell. Actively modifying intracellular structures allows cells to change and adapt to different conditions. One of these cellular structures is the microtubule cytoskeleton, which is comprised of polarized filaments that function as tracks to transport cargo via molecular motors. Recent studies have revealed the importance of cooperative transport in living cells. In the moss Physcomitrella patens, reorganization of the chloroplasts is critical to adapt to changes in light quality and intensity. In this work, we performed detailed analysis of the transport of chloroplasts in protonemal moss cells, and showed that their transport is facilitated by molecular motors and the microtubule cytoskeleton. Our findings were recapitulated using coarse-grained modeling of this cargo transport over different microtubule network topologies. Our simulations include a detailed description of motor and microtubule dynamics, in the presence of thermal fluctuations. Our modeling sheds light into the molecular mechanisms at play in the photo-relocation response of chloroplasts in moss.

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Logistics of Intracellular Transport Required for Cell Wall Assembly

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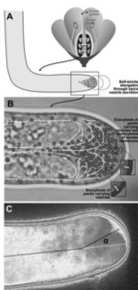
In plants, cellular growth requires the assembly of extensive amounts of new cell wall surface. The targeted deposition of building material through exocytosis - cell wall polymers, enzymes and membrane material - is therefore a crucial regulatory feature in plant development. The spatial and temporal regulation of the delivery of cargo vesicles to the target surfaces are poorly understood. Spatio-temporal image correlation spectroscopy (STICS) was used to quantify the intracellular dynamics of secretory vesicles and of the actin arrays in pollen tubes - rapidly and polarly growing plant cells. The dynamic profiles were used to validate mathematical models for vesicular trafficking. Boundary conditions were the expanding cell wall (Fig.1A) and the actin array whose shape was obtained by imposing a steady state and constant polymerization rate of the actin filaments (Fig.1B). The model correctly predicted the vesicle flow patterns in different types of pollen tubes and provides an explanation for flow dynamics in cellular regions devoid of actin cytoskeleton. It will serve as a basis for understanding how pollen tubes are able to regulate their morphogenetic pattern, for example when responding to a directional trigger by changing the growth direction (Fig.1C).



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Understanding Non Brownian Dynamics of Intracellular Transport

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Understanding insulin granule transport in live beta cells is a complicated task. Traditionally, the diffusion coefficients and the velocity of insulin granules measured via particle tracking techniques are used to characterize the dynamics, which requires the assumption that the dynamics to be either purely diffusive or ballistic. This is not the case for insulin granules. We use a variety of statistical data analysis, to show that insulin granule vesicles in their pathway, which leads to exocytosis performs a subordinated intercellular transport mechanisms, which leads to a statistical anomalous dynamics.

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Regulating Intracellular Transport with External Stimuli

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Cellular physiology and metabolism depend on mechanisms that regulate the transport macromolecules and organelles along cytoskeletal filaments. In many cases, these cargos are transported by multiple motor proteins that work either agonistically or antagonistically to control the directions of cargo motion. A number of new experimental techniques have been developed to study how the interactions between motors affect cargo transport, examining these problems inside of living cells has been much more challenging. The main aim of this study is to develop an intracellular assay that facilitates examination of mechanism governing cargo transport by multiple motors. To do so, we have designed cell lines that allow the coupling an uncoupling of motors to and from cargos via the application of an external stimuli. These cells provided control over the number of cargo attachment sites and the number of motors that are attached to these sites. The use of this system to examine the cooperation and competition of motors will be discussed.

1924-Pos Board B694

Mapping Vesicle Trafficking during Plant Cell Cytokinesis using Spatio-Temporal Image Correlation Spectroscopy

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The delivery of new cell wall material to the forming cell plate of a dividing plant cell requires intricate coordination of secretory vesicle trafficking. The vesicles need to be transported rapidly and efficiently to precise locations in the cell at specific times in order for cell division to occur normally. The trafficking of vesicles is mediated by the cytoskeleton via complex regulatory mechanisms. However, the dynamics of the vesicle delivery is difficult to measure in living cells due to their small size and high density. The vesicle dynamics are measureable via Spatio-Temporal Image Correlation Spectroscopy (STICS), a fluorescence fluctuation method that was initially developed to measure the directed transport or flow of proteins inside living cells. STICS relies on calculating the complete space-time correlation function of the intensity fluctuations between images of a time series obtained using a fluorescence microscope. Here, we use STICS to analyze laser scanning confocal microscopy image time series to obtain quantitative information on secretory vesicle dynamics in plant cells between their production from Golgi stacks and the final step of vesicle docking and fusion at the cell plate initiation site. We were able to map the direction and magnitude of vesicle movement at the different stages of cell division. This allowed us to determine the range of velocities of vesicles and to observe the varying flow patterns and the fast changing nature of their dynamics during the formation of the cell division plate.

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Intracellular Trafficking of Lipid Gene Vectors Investigated by Three-Dimensional Single Particle Tracking

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Three-dimensional single particle tracking (SPT) was applied to investigate the intracellular trafficking of multicomponent (MC) lipoplexes in CHO-K1 cells. In untreated (NT) cells, we have found that: (i) intracellular lipoplex motion was either directed or Brownian;(ii) the occurrence of directed motion was more frequent (more than 70%) than the Brownian one;(iii) within experimental error, the Brownian motion (D~0.7•10-3 um⊥2/s) was faster than the directional movement (D~0.35•10-3 um⊥2/s);(iv) the directed motion mean velocity was about v=0.032 um/s;(v) the calculated three-dimensional asphericity, A3, was close to unity denoting the privileged occurrence of movement along a direction. To elucidate the role of the cytoskeleton structure in the lipoplex trafficking, cells were treated with cytoskeleton (actin microfilaments and microtubules) polymerization inhibitors (Latrunculin B and Nocodazole, respectively). In inhibitor-treated cells, we have found that: (i) the percentage of directional movement decreased balanced by the simultaneous increase in the occurrence of Brownian motion;(ii) reduction of directional movement was large but never complete. Such observation might reflect either an incomplete disruption of cytoskeleton network by drug treatment and/or its recovery due to the kinetic profile of the drugs employed;(iii) the effect of Nocodazole on the reduction of directional movement was definitely stronger than that of