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When size does matter: organelle size influences the properties of transport mediated by molecular motors

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ABSTRACT

Background: Organelle transport is driven by the action of molecular motors. In this work, we studied the dynamics of organelles of different sizes with the aim of understanding the complex relation between organelle motion and microenvironment.
Methods: We used single particle tracking to obtain trajectories of melanosomes (pigmented organelles in *Xenopus laevis* melanophores). In response to certain hormones, melanosomes disperse in the cytoplasm or aggregate in the perinuclear region by the combined action of microtubule and actin motors.
Results and conclusions: Melanosome trajectories followed an anomalous diffusion model in which the anomalous diffusion exponent (α) provided information regarding the trajectories' topography and thus of the processes causing it. During aggregation, the directionality of big organelles was higher than that of small organelles and did not depend on the presence of either actin or intermediate filaments (IF). Depolymerization of IF significantly reduced α values of small organelles during aggregation but slightly affect their directionality during dispersion.
General significance: Our results could be interpreted considering that the number of copies of active motors increases with organelle size. Transport of big organelles was not influenced by actin or IF during aggregation showing that these organelles are moved processively by the collective action of dynein motors. Also, we found that intermediate filaments enhance the directionality of small organelles suggesting that this network keeps organelles close to the tracks allowing their efficient reattachment. The higher directionality of small organelles during dispersion could be explained considering the better performance of kinesin-2 vs. dynein at the single molecule level.

1. Introduction

Molecular motors transport a wide variety of cellular components positioning them in the cytoplasm with high spatial–temporal precision. These proteins bind to specific cargoes and step along cytoskeletal filaments (i.e., microtubules or actin filaments) using energy provided by ATP hydrolysis. Biophysical properties of molecular motors have been extensively studied by single molecule/particle techniques which provided extremely valuable information in in vitro systems [1] and in living cells [2–5].

Organelle transport is driven by 3 families of motors: dynein and kinesin, which transport cargoes toward the minus and plus ends of microtubules, respectively, and myosin, responsible for the transport along actin filaments (reviewed in [6,7]). Although these motors allow motion of micrometer-sized organelles through the cytoplasm, we still do not completely understand how organelles move in the complex cellular environment in which other active and passive forces seem to play a very important role. In this context, Kulic et al. [8] studied the motion of peroxisomes along microtubule tracks and demonstrated that the movement of microtubule filaments also drives the motion of organelles. Similarly, Semenova et al. [9] proposed that the dynamics of actin filaments is essential for myosin-based transport. These studies clearly showed that the cytoskeleton cannot be only considered as static tracks.

Despite the observation that intermediate filaments are not directly involved in transport driven by molecular motors, several works showed that this network greatly affects organelle motion. Kural et al. [10] showed that disruption of the intermediate filament network in melanophore cells results in faster transport of pigmented granules along microtubules and shorter duration of the steps of the myosin V motor during melanosome transport. These authors also suggested that intermediate filaments physically hinder melanosomes by increasing

Abbreviations: IF, intermediate filaments; MSD, mean square displacement; OR, optical radius; α, anomalous diffusion exponent; FE-SEM, field emission-scanning electron microscopy; DLS, dynamic light scattering

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the viscous drag. More recently, Chang et al. [11] showed that vimentin filaments form cage-like structures surrounding single or small clusters of melanosomes. This last study revealed that the number of moving organelles and their speed as well as their processivity increase significantly when the intermediate filaments network was disrupted. Since purified melanosomes contain a substantial amount of the intermediate filament protein vimentin, these authors suggest that melanosomes bind intermediate filaments.

Other cellular cargoes such as mitochondria are also slowed down by the intermediate filament network [12]. However the role of this cytoskeletal network on cargo mobility cannot be generalized to every cellular system. In this sense, Potokar et al. [13] explored the dynamics of single vesicles labeled with fluorescent atrial natriuretic peptides in rat astrocytes and observed that the fraction of vesicles presenting directional motion decreases in the absence of intermediate filaments.

Although, previous studies described some effects of the cellular environments on organelle transport, the low temporal resolution of those studies does not allow getting precise information about the local motion mechanisms of organelles.

Xenopus laevis melanophores are one of the most widely used cellular systems for the study of intracellular transport [14]. These cells have organelles called melanosomes, which contain the black pigment melanin and thus they can be easily observed using brightfield microscopy. Melanosomes distribute in cells in two configurations: either aggregated within the perinuclear region or homogeneously dispersed through the cytoplasm. Transport of pigment organelles during aggregation and dispersion is regulated by signaling mechanisms initiated by the binding of specific hormones to cell surface receptors, which results in the modulation of cAMP concentrations [15,16]. Organelles are transported along microtubules by the action of cytoplasmic dynein [17] and kinesin-2 [18] while myosin V is responsible for actin dependent transport [19].

In this work we used a fast and precise single particle tracking method to follow the motion of individual melanosomes in the cell cytoplasm of *X. laevis* melanophores and analyzed their trajectories to obtain information regarding the relation between organelle dynamics and local microenvironments during both the aggregation and dispersion processes. We found that small organelles, which are supposed to experience a smaller drag force, present more tortuous trajectories and found that the actin and intermediate filament networks play important roles in this behavior.

2. Materials and methods

2.1. Cell culture and sample preparation for imaging

Immortalized *X. laevis* melanophores were cultured as described in [20]. In order to track the movement of individual organelles, the number of melanosomes in cells was reduced by treatment with phenylthiourea [21]. For microscopy measurements, cells were grown for 2 days on 25-mm round coverslips placed into 35-mm plates in 2.0 ml of complete medium. Before observation, the coverslips were washed in a serum-free 70% L-15 medium (Sigma-Aldrich) and mounted in a custom-made chamber specially designed for the microscope. The cells were stimulated for aggregation or dispersion with 10 nM melatonin or 100 nM MSH, respectively. Actin depolimerization was achieved by incubation of the cells with 10 mM latrunculin B (Sigma-Aldrich) for 30 min before hormonal stimulation. Samples were observed during 15 min after stimulation.

2.2. Plasmids and tranfection

Cells grown on coverslips were transfected using Lipofectamine 2000 (Invitrogen) following the vendor instructions and observed 24 h after transfection. Two different plasmids were used: a GFP-tagged full-length *Xenopus* vimentin, which co-assembles with endogenous vimentin, and the dominant-negative construct containing the head and alpha-helical domain 1A of vimentin [GFP-vim(1-138)] that disrupt the endogenous vimentin filament network [11]. Both plasmids were a kind gift from Dr. Vladimir I Gelfand (Northwestern University, Chicago, IL).

2.3. Melanosome purification and scanning electron microscopy measurements

Melanosomes were purified and fixed onto a coverslip as described in [20,22]. In order to precisely locate every observed melanosome, SEM Au grids 400 mesh (Electron Microscopy Science, USA) were carefully glued at their ends to the coverslip and subsequently observed in the optical microscopy used for tracking measurement. Samples were then processed to be observed by field emission-scanning electron microscopy (FE-SEM). FE-SEM images were taken with a Zeiss Leo 982 Gemini microscope in the secondary-electron mode using an in-lens detector.

2.4. Nanoparticle synthesis and characterization

Gold nanoparticles were synthesized following the citrate method based in Turkevich's work [23]. Dynamic light scattering (DLS) measurements of Au nanoparticles dispersed in water showed particles with 22 nm hydrodynamic diameter with a PI = 0.20 (polydispersity index). DLS measurements were carried out in a Brookhaven BI-200 SM goniometer assembled with an avalanche photodiode detector and a He–Ne laser (wavelength = 637 nm).

2.5. Confocal microscopy

Confocal images were acquired in a FV1000 Olympus confocal microscope (Olympus Inc., Japan). EGFP fusion proteins were observed using a multi-line Ar laser tuned at 488 nm (average power at the sample, 700 nW) as excitation source. The laser light was reflected by a dichroic mirror (DM405/488) and focused through an Olympus UPlanSApo 60× oil immersion objective (NA = 1.35) onto the sample. The fluorescence was collected by the same objective, passed through the pinhole, reflected on a diffraction grating, and passed through a slit set to transmit in the range 500–600 nm. Fluorescence was detected by a photomultiplier set in the photon-counting detection mode. The pixel size was 131 nm.

Confocal imaging of C-Laurdan labeled cells was performed as described before [24] using a solid diode laser at 405 nm as an excitation source (average power at the sample, 2 μW). The laser light was reflected by a dichroic mirror (DM405/473) and focused onto the sample through the objective. Fluorescence of C-laurdan was collected by the objective, passed through the pinhole and split with a dichroic mirror into 2 independent spectral detectors set to simultaneously collect fluorescence in the range 415-455 nm and 490–530 nm (channels 1 and 2, respectively).

2.6. Tracking experiments

Single particle tracking experiments were carried out in a FV1000 Olympus confocal microscope adapted for SPT using a 40× or a 60× oil-immersion objectives (numerical aperture: 1.30 and 1.35, respectively). A high-speed electron-multiplying CCD camera (Cascade 128+, Photometrics, Tucson, AZ) was attached to the video port of the microscope for imaging the cells. Movies (2000 frames) were registered at a speed of 333 frames/s except where indicated. The accuracy on melanosome position determination was assessed by tracking melanosomes in a fixed sample and was found to be in the range 4–7 nm.