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Multiple Copies of Active Dynein or Kinesin Produce Discrete Melanosome Velocities in Xenopus Melanophores

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Organelle transport is essential for a variety of cellular functions. Xenopus melanophores have black pigment organelles or melanosomes which, in response to hormonal signals, disperse in the cytoplasm or aggregate in the perinuclear region. Melanosomes are moved by two microtubule motors kinesin-II and cytoplasmic dynein and an actin motor myosin V. One of the unanswered questions regarding organelle transport *in vivo* is whether a single or multiple copies of the motor protein are necessary for transporting an organelle. In this work, we explore this question by using a new fast-tracking routine designed for a microscope under bright field illumination which allows us to determine the melanosome position in a cell every 10 ms with 2 nm precision. By using this approach, we measured the velocity distribution of melanosomes transported by dynein or kinesin under conditions of aggregation and dispersion. In order to eliminate the effects of non-microtubule component of the transport, actin filaments were depolymerized by latrunculin B. Analysis of approximately 200 melanosomes trajectories in multiple cells produced experimental velocity distributions. These distributions presented several peaks and could not be fit with a single Gaussian function. We postulated that the melanosome velocity depends linearly on the number of active motors dragging the organelle. This model satisfactorily fit the experimental data. According to the fitting results, 1 to 4 dynein transport melanosomes during aggregation. In the case of melanosomes dragged by kinesin, the transport toward the plus end is mainly due to the action of 1 to 3 motors. The number of active dynein motors decreases during melanosome dispersion. The distribution of active kinesin bound to melanosomes does not change after inducing aggregation or dispersion. Supported by the NIH PHS 5 P41-RRO3155, and NIH PHS GM-52111.