Vesicle transport: ***klarsicht* clears up the matter**

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Lipid droplets can be seen to move around on microtubule tracks in a characteristic manner within a developing *Drosophila* embryo. This phenomenon has allowed genetic studies to be combined with biophysical measurements of single moving droplets, providing a clear view of motor protein control *in vivo*.

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Current Biology 1998, 8:R542–R544
http://biomednet.com/elecref/09609822008R0542

The transport of organelles and protein complexes to defined intracellular locations is fundamental to the specification, morphogenesis and working of eukaryotic cells. Early investigations of particle transport in axons revealed that membranous organelles move by fast flow in both directions [1–3]. It soon became clear that such movement is dependent on tube-like tracks: microtubules, linear polymers of αβ tubulin heterodimers. The two ends of a microtubule, 'plus' and 'minus', differ with respect to morphology and function; tubulin subunits are added only to the plus end during microtubule growth. Microtubules are interconnected by various associated proteins to form a regular lattice within the cell. Every eukaryotic cell contains characteristic patterns of microtubule tracks that undergo dynamic changes during the differentiation or working of the cell [2].

Movements of organelles along these tracks are mediated by motor proteins [3]. Motor proteins include members of the kinesin and dynein superfamilies [3]. As a general rule, kinesins mediate transport towards the plus end of microtubules, whereas dyneins mediate transport towards the minus ends. *In vitro* studies using isolated microtubules have revealed many of the transport characteristics of kinesins and dyneins. Kinesins appear to maintain a tight grip on the microtubule, as cargoes transported by kinesins display little or no side-to-side motion [4,5]. In contrast, dyneins move more freely towards the minus poles, resulting in lateral motions of the transported cargo [6]. Both types of motor power the transport of a variety of distinct cargoes, such as chromosomes, pigmented granules, mitochondria and other organelles.

Despite our detailed knowledge about the structure and function of motor proteins [3], we still do not understand how cells manage to target particular cargoes to specific intracellular sites at specific times. In an elegant recent study, Welte *et al.* [7] have established an *in vivo* model system, in which they investigated the coordinated switching from one transport mode to another using genetic and biophysical methods. Using flattened, but otherwise intact, *Drosophila* embryos, they were able to show that the bulk movement of lipid droplets during early embryogenesis is tightly controlled, and that many individual droplets obey this control simultaneously.

Movement of the lipid droplets was easy to visualize, and both the kinetics of single moving droplets and the forces they generate during movement were monitored. Welte *et al.* [7] observed that, every now and again during development, the direction of droplet movement changed simultaneously in a characteristic manner, the microtubule network remaining unchanged. They also used their system to look at embryos affected by the mutation *klarsicht* — German for "clear view" — which has been exploited in the past to generate transparent 'photogenic' embryos [8]. The mutation appears to cause a defect in the switching mechanism, thus offering a first glance at the still enigmatic mechanisms that control the direction of microtubule-based cargo transport.

Developmental control of droplet movement

The distribution of lipid droplets in the *Drosophila* embryo changes dramatically during development [9], and three distinct phases can be distinguished. Phase I corresponds to the precellular blastoderm stage of embryogenesis, when the droplets are localized in the cortex at the periphery of the embryo. During phase II, when a cellular blastoderm has formed, the droplets are cleared from the cortex and accumulate basally, surrounding the central yolk. Phase III begins at the onset of gastrulation, when the distribution of the droplets throughout the peripheral cellular layer changes from basal to apical. Because the lipid droplets are large enough to cause light scattering, their bulk movements into or out of the cortex cause global changes in the opacity of the embryo, from cloudy to clear at the phase-I-to-phase-II transition, and back again to cloudy at the phase-II-to-phase-III transition. Although the total number of droplets varies somewhat from embryo to embryo, their number does not appear to change with developmental time. Thus, the changes in opacity are caused by the movement of preexisting droplets.

Careful analysis has shown that the changes in droplet distribution are not caused by polymerization or 'treadmilling' of microtubules. Firstly, the tracks along which the droplets move coincide with basal–apical microtubule extensions. Secondly, a kinesin–β-galactosidase fusion protein [10] redistributes from an initially uniform pattern

to a distinct zone below the cortex, suggesting that the microtubule track extensions remain constant. Thirdly, droplet motion ceases during syncytial mitosis, when cytoplasmic microtubules are disassembled prior to spindle formation. Fourthly, drugs that destabilize microtubules abolish the movements almost immediately. And finally, the polymerization rate of microtubules, about 20–30 micrometers per minute [11], is too slow to account for droplet movements, which have a speed of about 60 micrometers per minute. The changes are thus mediated by motor proteins that move the droplets along preexisting microtubule tracks.

Kinetics of droplet motion

Welte *et al.* [7] were able to visualize and trace individual lipid droplets at nanometer scale resolution, and thereby determine whether the developmental changes in opacity of *Drosophila* embryos are caused by droplet movements. The investigators flattened embryos between a cover glass and a slide, and tracked droplet movement on a time-lapse video recorder using differential interference contrast microscopy. They found that lipid droplets move bidirectionally and in a saltatory fashion. These movements are very similar to those of organelles in axoplasm, which are known to move in an irregular and saltatory fashion on microtubule tracks. Occasionally a droplet jumps between tracks and changes direction. Given this apparently random behavior at the microscopic scale, how is directed and coordinated movement of large droplet populations achieved?

To answer this question, Welte *et al.* [7] quantitatively analysed the movements of many droplets over defined periods of time. They recorded three particular parameters: the time spent travelling in either plus-end (basal) or minus-end (apical) directions; the distance travelled in either direction; and the velocity maintained during travel. These parameters enabled them to calculate average net movements during defined developmental stages. For example, during a 7.1 second interval in phase II, the mean distances travelled by droplets were 1092 nanometers apically and 1495 nanometers basally, resulting in a net translocation towards the basal pole of 403 nanometers, with a bulk net speed of about 3.4 micrometers per minute. The net average fluxes computed from the tracking data for many individual droplets correctly predict the direction of the bulk changes: minimal shifts during phase I, inverted displacement during phase II and outward displacement during the clouding in phase III. Interestingly, the speeds travelled in either apical or basal direction were largely constant. The differences in bulk movement were mainly due to changes in the average time periods the droplets spent travelling towards the basal pole.

Is the net movement of droplets genetically controlled? The *klarsicht* mutant [8] provided an answer to this question. Disruption of components involved in movement control should alter the characteristic pattern of droplet motion, recognizable by opacity in affected embryos. In *klarsicht*, a maternal-effect mutation, embryos are unusually transparent from gastrulation onwards, as the lipid droplets remain concentrated below the cortex. When droplet motion was analyzed in *klarsicht* mutant embryos, their movements were seen to be less frequent and less vigorous than in wild-type embryos. Furthermore, the basal and apical velocities and travel distances of single droplets were less than half those of wild-type embryos, and the average time spent travelling towards the plus (basal) pole was significantly prolonged during all three phases. The lack of clouding during phase III in *klarsicht* mutants can thus be explained by a net basal flux of lipid droplets. The polarity of microtubule tracks appears to be undisturbed, however, as the kinesin reporter construct accumulated below the cortex just as in wild-type. One can therefore conclude that *klarsicht* affects lipid droplet transport by means other than by interfering with the microtubule network.

How about motors?

Infrared-laser-based optical traps, so-called 'optical tweezers' [12], have been used to measure the forces produced by single motor proteins *in vitro*. Welte *et al.* [7] applied a variation of the 'escape force' method to their embryo preparation [12,13]. The forces were set in such a way that only a fraction of the droplets was trapped by the laser. Droplets that were powered by forces higher than the trap setting escaped. The relationship between laser power and the proportion of escaped droplets can be used to calculate the mean stalling force for droplet motion. In wild-type embryos, this force changed from 3.3 picoNewtons in phase I to 5.5 picoNewtons in phase II and 4.7 picoNewtons during phase III. Interestingly, no significant differences were observed for droplets escaping in apical or basal directions. In contrast, low forces of about 1.2 picoNewtons were observed during all phases and in both directions in *klarsicht* mutant embryos.

The best way to explain these distinct force changes is that they are caused by alterations in the number of motor proteins powering the motion of an individual droplet. The mean stall forces appear to be discrete multiples of about 1.1 picoNewtons, and it is plausible to assume that this is the force generated by a single motor molecule or motor unit. If this is so, then in wild-type embryos phase I droplets are powered by three active motor units, phase III droplets by four and phase II droplets by five. The droplets in *klarsicht* mutants would then be driven by only one motor unit. As the unitary force of ~1.1 picoNewtons differs from 4–6 picoNewtons found for kinesin *in vitro* [14], the motors driving lipid droplet movements may be either less powerful or less effective *in vivo* than *in vitro*. Alternatively, they may be of a different nature.

Droplets in wild-type embryos showed consistently larger side-to-side movements when travelling towards the minus ends of microtubules than when travelling in the opposite direction. The simplest explanation for this phenomenon is that the apical-end-directed motors belong to the dynein protein family, whereas the basal-end-directed motors are kinesin-related proteins. In *klarsicht* mutant embryos, however, the lateral displacements were smaller than those observed in wild-type embryos and were not different for motion in the apical and basal direction. This leaves open the possibility of a bidirectional motor unit, provided that its properties change sufficiently with directional polarity in wild-type embryos.

The model

The results of Welte *et al.* [7] show that motor-driven droplet movement is switched from one transport mode to the next, and that this switch is implemented simultaneously throughout the entire embryo. The observations indicate that the motor proteins are globally coordinated, even though their precise nature is not yet known. The *klarsicht* mutant embryo provides a clear view of what happens when the switching mechanism is damaged. It would seem that the *klarsicht* gene product is somehow involved in organizing a coordinated complex of motors on the droplet surface, thereby avoiding a tug-of-war that might otherwise ensue from the simultaneous action of opposite-direction motor proteins. How this is achieved remains to be investigated.

The wild-type Klarsicht protein might control the number of actively engaged motors on a droplet, and switch off the basal-end-directed motors when apical-end-directed motors are active, and *vice versa*. This switch could be achieved, for example, by presenting either plus-end-directed or minus-end-directed motors to the microtubules, or by coupling several bidirectional motors so that they alter directions synchronously. Alternatively, Klarsicht's role might be to recruit the appropriate motor protein to the cargo, thereby determining the direction of transport. The Klarsicht–motor complex is the most likely candidate for a developmental control that alters the frequency of switching and thus affects the time of travel in one direction which, as we have seen above, determines the direction of net cargo transport.

Perspectives

What makes the study of lipid droplet transport in *Drosophila* embryos so interesting is that, for the first time, microtubule-dependent organelle movement has been studied in an *in vivo* system that is easy to visualize and accessible to biophysical manipulations. The most enlightening feature of this model system is contributed by *klarsicht* itself: it provides the first clear view of the complex regulation of microtubule-dependent transport. The power of genetics can now be exploited to identify factors and molecular pathways that control motor-based transport systems.

Squeezed *Drosophila* embryos, in conjunction with the excellent *in vitro* systems established for microtubule-based transport, will provide the tools to test individual components of the transport machineries and to learn how they are integrated into cellular pathways and whether and how they respond to external signals. Interestingly, the *klarsicht* mutation not only interferes with droplet movement but also with the transport of nuclei of photoreceptor cells as they mature during eye development. How important is this observation? It demonstrates multiple roles for *klarsicht*-dependent transport implying a high degree of redundancy and complexity for which a solution is also made transparent by *klarsicht*: genetics is one way to move!

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