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Intracellular Transport: How Do Motors Work Together?

How many motors move cargos on microtubules inside a cell, and how do they work together to achieve regulated transport? A new study uses an optical trap to investigate the motion of protein-bound beads on the surface of flagella to address these questions and comes up with some intriguing answers.

Roop Mallik[1] and Steven P. Gross[2]

The study of cytoskeletal molecular motor-driven transport has come a long way. Not so long ago, the focus was on single motors and their properties, but new studies from several groups have highlighted the more complex nature of the transport problem. Multiple motors move cargos and, in many cases, motors of opposite polarity are attached simultaneously, so that a specific cargo can in principle move in either direction [1–5]. This raises the as yet unanswered questions of how do motors function together, and how is net transport controlled? A recent publication [6] in *PNAS* develops a powerful system — flagellar surface motility — that is amenable to both biophysical and genetic approaches and reveals intriguing similarities and differences with other bi-directional transport systems. Understanding conserved and unique aspects of this system will likely lead to a deeper understanding of intracellular transport.

The absolute number of motors moving a cargo is likely to influence transport — experiments *in vitro* show that two or three kinesin or dynein motors move cargos much further than one [7,8]. The relative concentration of opposing motors is also important because this can bias transport in either direction. However, it is not so easy to determine the number of engaged and active motors by standard biochemical techniques because some cargo-bound motors may be inactive. Biophysically, one way to do this is by measuring the force required to stop cargos, since, at least for small numbers of motors, motor stall forces are approximately additive [7–9]. Such stalling force measurements are relatively straightforward *in vitro* where well-characterized polystyrene beads coated with motors are used in buffer. However, calibrated force measurements *in vivo* are technically challenging because endogenous cargos vary in size and move in cytoplasm of unknown properties.

The new system of Laib *et al.* [6] is clever in that it makes possible such stalling force measurements by combining some of the best aspects of both *in vitro* and *in vivo* studies. In short, an intact living *Chlamydomonas* cell is affixed to a coverslip, and its flagella are immobilized. Then, when a laser trap is used to bring a microsphere (the cargo) into contact with the flagellum (Figure 1), the microsphere binds to the flagellar plasma membrane, specifically to the FMG-1 flagellar membrane protein, and is subsequently transported along the flagellum in either an anterograde or retrograde manner by molecular motors inside the flagellum that are coupled to FMG-1. Thus, the microsphere is *in vitro* (bead in buffer) but it is expected that it reports on the action of motors *in vivo* (moving inside the intact flagellum). Once the microsphere binds and starts to move, its position is measured with a laser/quadrant diode system with very high temporal and spatial resolution.

To measure the force applied by motors, the bead's motion (opposed by the optical trap) is monitored; the maximal displacement of the bead from the center of the trap (~80 nm) is then multiplied by the trap stiffness to calculate the maximal force (~60 pN) applied by the motors. The measurements of Laib *et al.* [6] cannot resolve the forces of single dynein or kinesin motors in this system. Instead, the forces they measure are interpreted to arise from around ten active motors in each direction with an assumption of ~6 pN for both kinesin and dynein motors. These measured forces and the inferred motor numbers are, surprisingly, significantly different from other *in vivo* force measurements of smaller internal vesicular cargos, such as mitochondria [10] and lipid droplets [11], each of which reported typical forces of less than 10 pN, reflecting fewer motors.

Since previous work found that beads only move a few microns even when no trap was present [12], it was surprising that so many motors appear to move the beads — beads driven by more than three or four motors *in vitro* move hundreds of microns. Three models were considered to account for these observations. The first hypothesizes a complex of kinesin, dynein and regulatory proteins (similar to a previous suggestion for lipid droplets [13]), able to disengage one set of motors and then rapidly engage the other set. This model appears consistent with all the data. The second, a 'biased accumulation' model, hypothesizes that signaling causes the FMG-1 membrane patch to become transiently 'sticky' to one set of passing motors; when the signaling changes, the motors detach, and motion in that direction ceases. While formally possible, this model requires a huge flux of moving motors to rapidly bind to the membrane patch (since pauses between reversals of direction only last for hundreds of milliseconds), which seems unlikely. Further, it would be inconsistent with other bi-directionally moving cargos where both sets of motors are bound to the cargo simultaneously [2]. The

third hypothesis is related, suggesting that the FMG-1 membrane patch is non-specifically sticky to pre-existing clusters of around ten moving motors, and motion ensues when one such cluster binds stochastically to the patch. This hypothesis also raises issues: what keeps such motors clustered? Wouldn't clusters interfere with each other? Two clusters of opposing directionality (ten dyneins in one, ten kinesins in the other) seem likely to jam up transport on the microtubule. Finally, why would runs end so rapidly? A cluster of ten motors should move long distances unless it detaches from the cargo, but the attachment appears quite robust — a force of approximately 60 pN is required to stop the cargos. Further work is clearly required to explore these possibilities more fully.

Regardless of the mechanism, the process can disengage one set of many motors (and then re-engage the opposing set) quite rapidly, within hundreds of milliseconds. Further, rapid inactivation of kinesin (via a temperature-sensitive mutant) does not immediately alter minus-end transport: dynein-mediated runs are not longer or more frequent. This observation, combined with the observation of a temporal separation between runs of opposing polarity, supports the hypothesis that the reversal process is able to turn off one set of motors and turn on another set independently. This suggestion is consistent with findings in many other systems (reviewed, for example, in [2]). Importantly, because of the pause between inactivation of one set and activation of the other, the findings from Laib *et al.* [6] are inconsistent with a recently proposed 'tug-of-war' model [3].

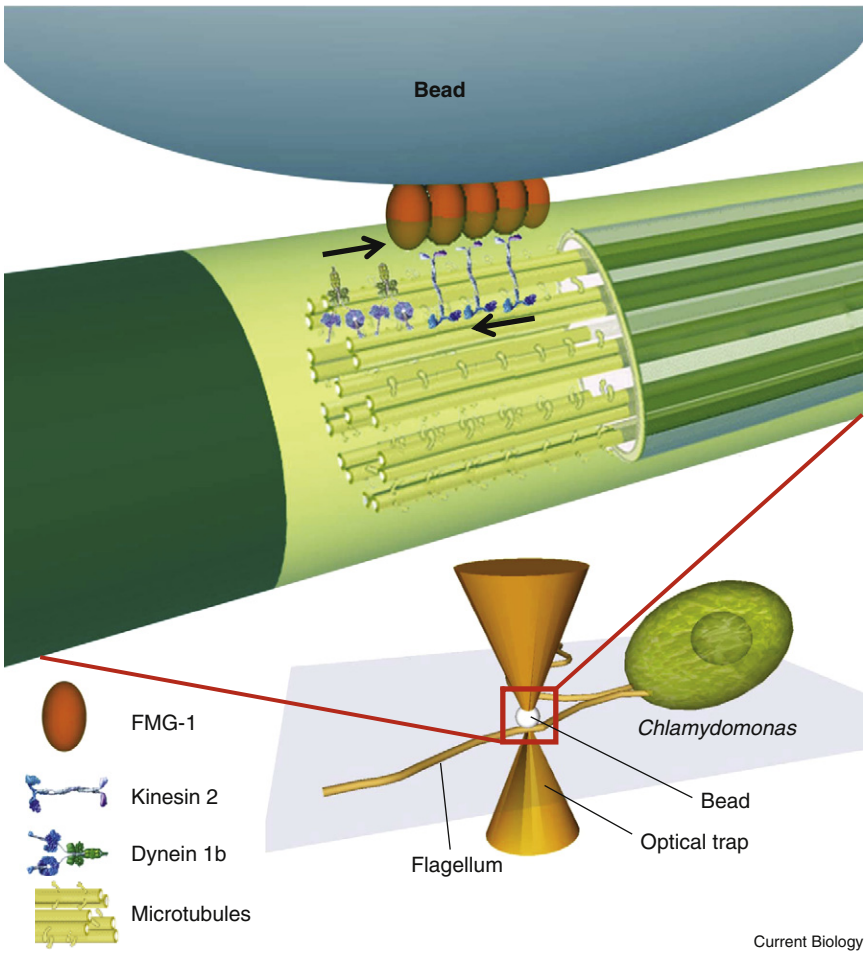

Figure 1. Schematic of the assay developed by Laib *et al.* [6] for the study of the transport of FMG-1-bound beads on the surface of a flagellum.

The flagellum of a single *Chlamydomonas* cell is immobilized on a coverslip, and a polystyrene bead held in an optical trap is lowered onto the flagellum. A cluster of FMG-1 proteins on the flagellar surface is believed to bind to the bead (red box; blown-up region). Back-and-forth surface motion of the bead thus represents FMG-1 motion driven by opposing microtubule motors (cytoplasmic dynein 1b and kinesin 2; arrows indicate direction of motion) within the flagellum. The optical trap exerts a controlled force ('load') on the bead (and connected motors), directed towards the trap center and proportional to the bead's displacement. Thus, the displacement is used to determine the force produced by the motors. Figure prepared with assistance from A. Ramaiya.

While the reversals thus have similarities with other bi-directionally moving cargos, there are also important differences. In addition to the difference in the number of engaged motors, in this system episodes of motion in a particular direction (and hence engagement of opposite polarity motors) are mutually exclusive and are temporally well separated. This is in contrast to vesicular transport, where opposite motors appear to engage in rapid succession (akin to an immediate switching mechanism, without a pause of hundreds of milliseconds), or simultaneously (as in a tug-of-war). The tug-of-war situation has been documented for mitochondria [14] and endosomes [15–17], while immediate switching occurs for lipid droplets [18] and melanosomes [4]. Given the similarities between some aspects of the reversal process for lipid droplets and the flagella–bead system, it is possible that the mechanism underlying the process is actually conserved, but the increased time between excursions in opposite directions results from the increased number of motors that must be engaged or disengaged in the latter case.

These similarities and differences with endogenous cargo motion raise interesting questions: is bead-attached FMG-1 transport really representative of actual transport of this protein? Given the large bead size, is there only one FMG-1 patch per bead? A second possibility is that the flagella–bead motion is unique and different from transport in cytoplasm of cells. However, it seems more likely that there are a number of different types of cargo transport, each adapted to specific requirements; if this is the case, the flagella–bead motion may be a 'founding member' of a new class of transport, or it may be an extreme, high-motor member of the lipid droplet motion family. It remains

for future work to explore these possibilities.

One very interesting observation, apparently conserved between multiple systems, is that the effect of a perturbation changes over time. The immediate effect of the temperature-sensitive kinesin mutant is to knock-out kinesin function rapidly but leave minus-end transport unaltered. However, over time, all transport ceases. This is interpreted to reflect the need for an anterograde motor to ship retrograde motors back out of the flagellum, but other forms of longer-term feedback are possible. For instance, in the lipid droplet case, similar to the temperature-sensitive effects described above, when a function-blocking anti-kinesin antibody is injected, kinesin function is selectively blocked, and there is net minus-end motion. Further, a kinesin-null mutant also blocks all minus-end motion driven by dynein [11]. However, when kinesin dosage is decreased by 50%, although droplet motion is unaffected from a transport point of view (i.e., the number of moving droplets is unaffected, and their travel distances and velocities are not decreased, and thus any effects cannot be due simply to an inability to come into contact with dynein), the number of engaged motors in both directions is decreased by 50% [11]. Thus, the observed longer-time impairment in the flagella case may also reflect subtle effects or feedback.

It is likely that the activity of opposing motors is regulated using different strategies for different classes of cargo. One of the immediate challenges facing the field is to determine how many such classes there are — is each type of cargo really different, or are there are a few general classes of cargo transport, each with its associated regulation of the underlying motors? At this stage we cannot arrive at a single general model of cargo transport, but the studies here develop an important new system that will help us approach this long-term challenge. Clearly, there will be a lot of back-and-forth before we understand how back-and-forth motion works in the cell.

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Functional Neuroanatomy: The Locus of Human Intelligence

A new study mapping the functional effects of brain lesions has revealed a surprising map of human intelligence, stimulating a re-evaluation of data from purely correlative methods such as functional magnetic resonance imaging.

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Where in the brain are the sites that determine intelligence? For some researchers, such a question is nonsensical: they would argue no single brain function could subserve something so complex. For others, though, the possibility of a discrete set of brain regions governing intelligence might not seem so bizarre [1]. Many behavioural studies show strong correlations between an individual's performance across a range of mental ability tests, pointing to a common, general factor [2,3].

But how do we find the neuroanatomical underpinnings of such a commonality? Because the defining feature of intelligence is the generality of its operations, there is no easy way of constructing a single set of tasks that uniquely isolates it. Most neuroanatomical studies have therefore focused on differences between people measured using a battery of mental tasks, exploring their putative biological basis using