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Lipid Rafts Assemble Dynein Ensembles

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Abstract

New work by Rai *et al.* identifies a novel mechanism regulating phagosome transport in cells: the clustering of dynein motors into lipid microdomains, leading to enhanced unidirectional motility. Clustering may be especially important for dynein, a motor that works most efficiently in teams.

The active transport of organelle cargoes along microtubules is essential for cellular survival and function. This transport, driven by the molecular motors kinesin and cytoplasmic dynein, may be unidirectional but is often bidirectional, characterized by frequent reversals and/or pauses. Microtubules are polarized tracks, with kinesins driving anterograde motility toward microtubule plus ends at the cell periphery and dynein driving retrograde, minus-end-directed movement toward the cell center. Accumulating evidence suggests that changes in the direction of cargo movement, or modulation between unidirectional and bidirectional states, is not due to gain or loss of opposing motors on the cargo being transported [1,2]. Instead, cargo-bound motors are regulated through such mechanisms as autoinhibition, post-translational modifications, and interactions with effector molecules [2,3]. A new study published in *Cell* by Rai *et al.* [4] proposes a novel geometric mechanism for motor regulation involving the lipid-dependent clustering of dynein motors into teams that work together to effectively compete against kinesin motors bound to the same cargo.

Phagocytosis of a particle such as a microbe or, in experimental systems, a latex bead results in the formation of a phagosome, in which the particle is encased in a membrane-bound organelle. Newly formed or early phagosomes (EPs) move in a bidirectional manner along microtubules while more mature late phagosomes (LPs) move unidirectionally toward the cell center; these movements are driven by associated kinesin and dynein motors. The relative forces generated by these motors can be measured in cells; moreover, the movement of these phagosomes can be reconstituted *in vitro* [4–6]. Previous work has shown that multiple dyneins are required to effectively oppose just a few kinesins, as dynein is a comparatively weak and detachment-prone motor. These differences also reflect the underlying biophysical properties of these motors: multiple kinesins do not work especially

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well together, while the clustering of dyneins into teams greatly enhances force generation and motility [5,6]. However, the regulatory mechanisms that establish these dynein ensembles have remained unidentified.

In their new study, Mallik and coworkers demonstrate that the switch from the bidirectional motility of EPs to the unidirectional motility of LPs correlates with the appearance of lipid microdomains, or lipid rafts, in the phagosome membrane, as well as the clustering of dynein motors into these cholesterol-rich microdomains.

Importantly, effective dynein teamwork is lost following extraction of the cholesterol, supporting the hypothesis that dynein clustering enhances its ability to work effectively in teams. In studies suggesting broader implications for their findings, Rai *et al.* [4] demonstrate that a cell surface lipophosphoglycan (LPG) from the protozoan parasite *Leishmania donovani* acts to disrupt the cholesterol-rich microdomains observed on LPs and thus perturb both dynein clustering and motility. The authors suggest that the inability to effectively move LPs through the cell may contribute to the ability of the parasite to escape intracellular degradation and thus enhances pathogenesis.

These findings raise several interesting questions. For instance, does this mechanism contribute to the regulation of motility of other types of cellular cargo? This naturally leads to the question of what establishes and maintains the distributions of both lipids and motors on a given cargo. More broadly, these observations now require us to consider how the characteristic lipid composition and organization within the membrane of a given organelle might affect the activity of the associated motors. Moreover, what is the diffusion rate of endogenous motors in the lipid membranes of organelles in cells? For example, the distribution of dynein on EPs apparently remains uniform during motility rather than remodeling in response to cytoskeletal interactions or force production, as has been observed with synthetic lipid–motor conjugates. This suggests that membrane-bound motors on native organelles are anchored within the membrane, likely due to interactions with scaffolding proteins, but more work is needed to clarify this issue.

Rai *et al.* [4] suggest that sustained unidirectional motility of LPs promotes phagosome–lysosome fusion. To test this hypothesis, they use a LPG from *L. donovani*, which is known to disrupt phagosome–lysosome fusion, and show that it prevents both dynein clustering and sustained motility. It has previously been shown that enhanced association of phagosomes with dynein precedes phagosome–lysosome fusion [7]; here, Rai *et al.* [4] propose that the clustering of dynein induces processive unidirectional motility and that this unidirectional, as opposed to bidirectional, transport facilitates robust phagosome–lysosome fusion. This model parallels a similar process in neuronal autophagy. Nascent autophagosomes are bidirectional and undergo a switch to processive, unidirectional, dynein-driven transport during maturation due to the binding of a scaffolding protein [8]. Blocking the binding of a scaffolding protein prevents the switch to sustained retrograde motility and also inhibits the acidification of the compartment, likely due to decreased fusion with lysosomes [8]. How this block in fusion occurs in either case remains to be seen, but in general terms it appears that organelle motility facilitates effective fusion.

The take-home message from Rai *et al.* [4] is that the clustering of dynein motors into lipid microdomains is an efficient way to regulate motor function, allowing teams of dynein motors to simultaneously engage with the microtubule track during the transport of large cargo through the cell. As dynein motors function effectively in teams, clustering thus allows dynein to ‘win’ the tug of war with opposing kinesin motors and produce sustained unidirectional transport. Moreover, treatment of phagosomes with *Leishmania* (LPGs) disrupts lipid microdomains, reduces dynein clustering, and decreases organelle motility, suggesting that these microdomains may have a causal role in the organization of dynein clusters. Although lipid-based and clustering-dependent enhancement of motility has been shown previously for some myosin and kinesin isoforms [9,10], the efficiency with which dynein works in teams suggests that lipid-mediated clustering may be an important regulatory mechanism for this motor as well. Thus, this represents another layer of regulation of organelle transport, complementing canonical pathways involving regulation by kinase/phosphatase activity or binding of protein effectors [2,3]. Additional research to identify the contributions of each of these forms of motor regulation, as well as how these mechanisms interact, will be informative.

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