Some motor proteins cooperate better than others

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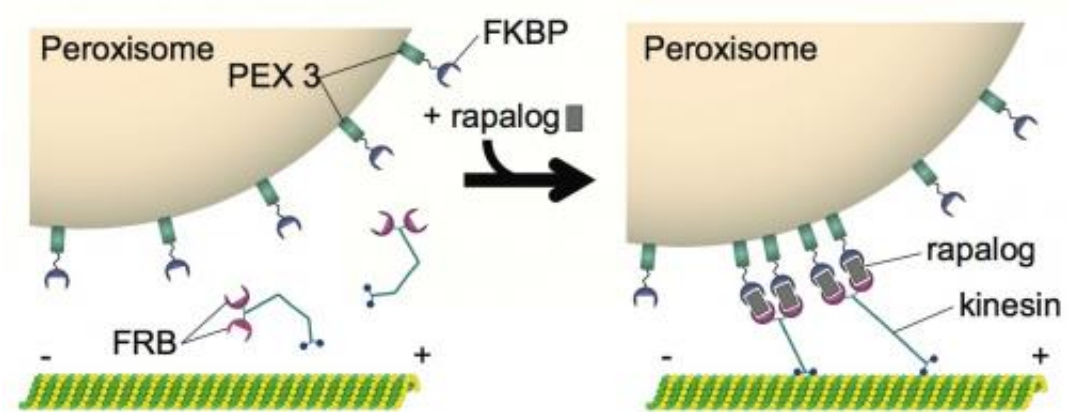


Scientists at Rice University regulated the size of vesicular organelles called peroxisomes and the density of protein motors on their surfaces to analyze how the motors cooperate as they move the vesicles inside cells. The PEX 3/FKBP receptors, when incubated with rapalog, allow transiently expressed kinesin and myosinVa motors to be coupled to vesicle surfaces via FRB protein fusions. Once coupled to the vesicles, the motors pull them along cytoskeleton filaments (bottom). Credit: Diehl Lab/Rice University

Rice University researchers have engineered cells to characterize how sensitively altering the cooperative functions of motor proteins can regulate the transport of organelles.

The study, by the Rice lab of bioengineer and chemist Michael Diehl, compared the collective behaviors of kinesin-1 and myosinVa in living

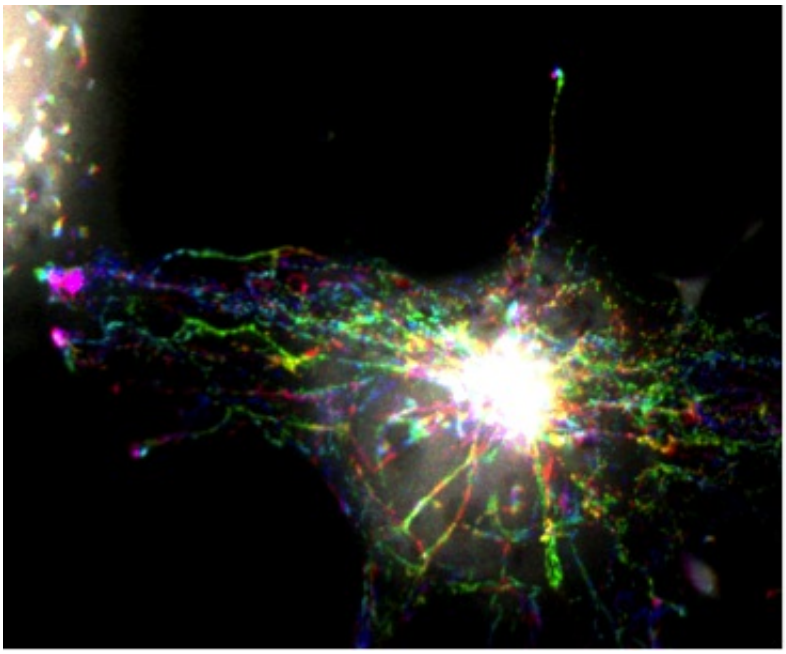
cells to determine how these motor proteins cooperate as they move vesicles and organelles along intracellular highways formed from cytoskeletal filaments. These transport processes are critical to numerous developmental and signaling functions within cells, and breakdowns in motor functions are also implicated in several human diseases.

The work appears this week in the *Proceedings of the National Academy of Sciences*.

Diehl and his colleagues at Rice's BioScience Research Collaborative, including Rice postdoctoral researchers Anand Radhakrishan and Artem Efremov and graduate student David Tsao, compared the collective responses of the motor proteins to variations in motor numbers and cargo sizes.

They began with a good understanding of the collective pulling power of kinesin motors. Kinesin is a type of protein that binds to and transports cargoes by walking along cytoskeletal filaments called "microtubules." In previous experiments, they engineered multiple motor systems that were anchored to polystyrene beads as an experimental cargo, but this time decided to engineer organelles called "peroxisomes" within living cells for these analyses.

"Our earlier work was detailed and very precise, but the central limitation was simply that the motors were not transporting a real cargo," Diehl said. In contrast to rigid beads, many organelles like peroxisomes have fluid-like lipid membranes. Motors attached to their surfaces will therefore interact in ways that are difficult to recapitulate using plastic beads.



Images by the Rice University lab of Professor Michael Diehl show peroxisome trajectories. After rapalog is used to trigger kinesin-peroxisome coupling, the peroxisomes are transported from the perinuclear space of the cell and towards the cell periphery. Credit: Diehl Lab/Rice University

"The physical environment inside a living cell is also difficult to emulate during in-vitro experiments," he said. "This could also have an appreciable impact on how motors cooperate to transport their cargoes."

Using genetically engineered COS cells, the team coupled motor proteins to peroxisomes via a certain type of protein switch. They regulated the expression of these protein switch genes in combination with a second gene that allowed them to tune the final density of motors on the surfaces of peroxisomes as well as the distributions of peroxisome sizes.

The genetic-level controls allowed the group to evaluate how the collective behaviors of kinesin and myosinVa motors responded to changes in motor levels and size-dependent forces imposed on the

peroxisomes by the cytoplasm. In contrast to behaviors found for kinesins, they show that the cargoes move more rapidly when myosinVa levels are altered and that myosin systems are more readily capable of producing the forces necessary to propel large cargos in living cells.

"Kinesins are like racehorses that basically only know how to run fast," Diehl said. "They don't like to work together in teams to transport cargos, and usually one kinesin is left doing the work of transporting a cargo on its own. MyosinVa motors, however, are more like stagecoach horses. If you add more horses, you get more force production. You get more speed. This distinction is important, since it suggests that collective functions of myosinVa can be regulated more sensitively than kinesins."

Kinesin and myosinVa are bound simultaneously to the surfaces of many organelles, Diehl said. The collective force-producing capacities and responses of cargo transport to variation in motor copy number will influence the trafficking of cargoes to different regions of cells. "Cells needs to be able to regulate that competition, and so it may be useful to have one motor, kinesin, that's strong individually but not capable of cooperating, and another motor that might be weak individually but have a strong cooperative effect," he said. "It means all the fine tuning can take place on one side."

The overall results open a new window into cellular mechanisms, Diehl said. "Now that we're able to probe detailed relationships between motor type, ratio, cargo size and force, we can start to examine more complicated collective and regulatory behaviors directly," he said. "We hope to recapitulate scenarios where multiple normal motors are bound to the surfaces of cargoes simultaneously with motor mutants that have been associated with neurodegenerative diseases. This way, we can precisely examine how these mutants perturb intracellular transport pathways."

More information: www.pnas.org/cgi/doi/10.1073/pnas.1313569111