


SPOTLIGHT

A CRACKer of an adaptor connects dynein-mediated transport to calcium signaling

Mahak Sharma  and Devashish Dwivedi 

Many different adaptor proteins activate the processivity of dynein–dynactin complexes and determine the specific cargo for retrograde transport by binding cargo receptors such as Rab GTP-binding (G) proteins. In this issue, Wang et al. (2019). *J. Cell Biol.* <https://doi.org/10.1083/jcb.201806097> identify two GTPases that can function directly as dynein adaptors during endocytosis and are regulated by calcium.

Cytoplasmic dynein-1 (dynein) drives the minus end-directed microtubule-based transport of a variety of cellular cargo, including subcellular organelles, small vesicles, mRNAs, proteins, and even viruses. A central question in the field is how dynein, the primary motor driving retrograde transport, achieves spatial and temporal specificity for interaction with these diverse cellular cargoes.

Unlike its yeast counterpart, mammalian dynein does not display processive motility by itself due to the auto-inhibited configuration of its motor domain (1). Association of dynein with the dynactin complex in the presence of adaptor proteins with coiled-coil domains induces large conformational changes in dynein, converting it into an ultra-processive motor with long-range motility (Fig. 1; 2). Such “activating adaptors” that have been discovered to date include BicD2, BicDL1, Spindly, Hook1, Hook3, Ninein, Ninein-like protein, and Rab11FIP3, which mediate dynein–dynactin assembly into a complex with robust processive motility (3). Notably, the activating adaptors also play a second important role, that of mediating dynein–dynactin recruitment to the cargo, by direct or indirect binding to a cargo receptor that is occasionally a Rab GTP-binding (G) protein (Fig. 1; 3). Rabs are a family of >60 small G proteins that localize to a specific intracellular compartment and mediate vesicular trafficking to and from these compartments. Notable examples of the Rab and activating adaptor associations

include Rab6 binding to BicD2 to mediate retrograde motility of Golgi-derived vesicles (4), Rab11 binding to Rab11FIP3 for transport of sorting endosomes toward the endocytic recycling compartment (5), and Rab5 binding to the FTS–FHIP complex, which in turn binds to the activating adaptor Hook1/Hook3 for retrograde transport of early endosomes (6).

In this issue, Wang et al. identify two remarkable and novel dynein adaptors, CRACR2a and Rab45, that combine the dynein-activating and cargo recruitment function into a single polypeptide (Fig. 1). Both proteins are Rab family members that have N-terminal EF-hand motifs followed by coiled-coil regions (similar to the dynein adaptors Rab11FIP3 and Ninein) and a Rab GTPase domain at their C-terminus. Prompted by the similarity to the known dynein adaptors (Rab11FIP3 and Ninein) and subcellular localization to a perinuclear compartment near the minus end of microtubules (7), the authors examined the role of Rab45 and CRACR2a as dynein adaptors. Both Rab45 and CRACR2a activated the processive motility of the purified dynein–dynactin complex on microtubule tracks, which was experimentally determined by total internal reflection fluorescence microscopy single-molecule assay (8). This assay, regarded as the gold standard for determining the activator function of a dynein–dynactin interaction partner, showed that the two large Rab GTPases, CRACR2a and Rab45, were indeed activating dynein adaptors (Fig. 1). Using a

rapamycin-induced heterodimerization approach, the authors artificially targeted Rab45 and CRACR2a to peroxisomes, which led to clustering of peroxisomes around the microtubule-organizing center (MTOC), thus confirming the “activating adaptor” role of Rab45 and CRACR2a in live cells as well (8).

The presence of calcium-binding EF-hands in both Rab45 and CRACR2a and prior observations suggesting a role for CRACR2a in calcium signaling (7) led the authors to explore whether the dynein association of these newly identified adaptors depends on available calcium concentration. Interestingly, CRACR2a, but not Rab45, showed an affinity for dynein–dynactin complexes only in the presence of calcium, and calcium binding-defective (EF-hand mutants) mutants of CRACR2a failed to interact with dynein (Fig. 1). Physiological elevation of the calcium concentration (i.e., from 10 nM to 2 μ M) significantly increased the processivity of dynein–dynactin–CRACR2a complexes on microtubules, further strengthening the notion that calcium-dependent CRACR2a binding to dynein is relevant under physiological conditions. To directly test this hypothesis, the authors next investigated the significance of calcium-dependent CRACR2A-mediated dynein motility in the Jurkat T cell line, which has high expression levels of endogenous CRACR2a. Indeed, increasing the intracellular calcium levels, either artificially

Department of Biological Sciences, Indian Institute of Science Education and Research Mohali, Punjab, India.

Correspondence to Mahak Sharma: msharma@iiser Mohali.ac.in.

© 2019 Sharma and Dwivedi. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).

Activating adaptors:

- stabilize and activate dynein-dynactin complex into a processive motor
- directly or indirectly bind to cargo receptors/Rab proteins to recruit dynein-dynactin to cargo vesicle

Gene fusion events have combined adaptor and membrane recruitment functions:

- activating adaptors Rab45 and CRACR2a contain Rab GTPase domain and can directly recruit dynein-dynactin to cargo vesicle

Regulation of adaptor binding to the dynein-dynactin complex by cellular factors:

- CRACR2a binds to dynein in the presence of calcium, thus linking cargo motility to calcium signaling

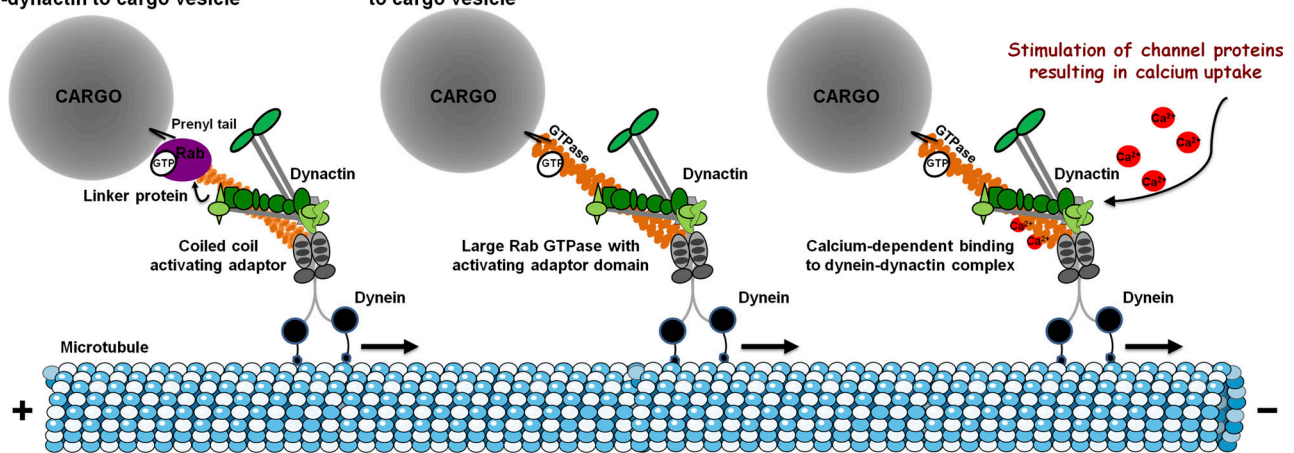


Figure 1. Evolving concept of activating dynein-dynactin adaptor proteins. Mammalian dynein requires binding to the dynactin complex in the presence of a coiled-coil domain-containing adaptor protein for processive motility on microtubule tracks. Additionally, the adaptor proteins also recruit dynein-dynactin to the cargo vesicle by direct or indirect binding to a cargo receptor, occasionally a Rab GTP-binding (G) protein (left). CRACR2a and Rab45 are the first example of Rab proteins that can directly act as dynein adaptors, combining the functions of dynein activation and recruitment to the cargo vesicle into a single protein (middle). Moreover, binding of CRACR2a to the dynein-dynactin complex is regulated by calcium, setting a paradigm for how activation of a signaling pathway can regulate dynein-mediated cargo motility (right).

using ionomycin (a calcium ionophore) or by anti-CD3 antibody-mediated T cell receptor (TCR) stimulation, resulted in calcium-dependent retrograde motility of CRACR2a-bound vesicles toward the MTOC (8). In future studies, it will be important to elucidate the identity of the CRACR2a-bound vesicles and significance of the Rab GTPase domain in dynein recruitment to these vesicular compartments.

Interestingly, Wang et al. (8) also observed the actin-dependent formation of smaller CRACR2a punctae near the cell periphery (cortical punctae) that were morphologically distinct from the large CRACR2a vesicles seen at the MTOC upon TCR activation. Notably, the CRACR2a-bound cortical punctae were colocalized with dynein light chain, and occasionally the authors observed switching of the slow-migrating cortical punctae to a fast retrograde movement on microtubules. The formation of CRACR2a punctae at the plasma membrane and their movement toward the MTOC prompted the authors to investigate a possible role of CRACR2a in endocytosis. When Wang et al. tested

different cell surface receptors/molecules that internalize through various endocytic routes, only CD47 internalized in a CRACR2a-dependent manner, suggesting it is a select cargo for CRACR2a-regulated calcium-stimulated endocytosis. CRACR2a has been previously shown to undergo GTP hydrolysis and be rapidly degraded in activated T cells (7), which begs the question whether both pools of CRACR2a-bound vesicles along with their cellular cargo are degraded upon TCR stimulation and what the significance is of dynein binding in this process.

In summary, Wang et al. (8) have identified dynein adaptor(s) with several unique and interesting features that include the presence of a Rab GTPase domain, calcium-dependent interaction with the dynein-dynactin complex, and the potential ability to mediate endocytosis of a cell surface molecule (Fig. 1). Gene fusions, as seen in this study, are one of the modes of protein evolution that not only increases the efficiency of coupling distinct cellular processes (such as dynein recruitment to target membranes and processive motility) but also provides tight co-regulation of expression of the fused protein domains.

Several questions remain unanswered: for instance, how common is the regulation of vesicle motility by signaling molecules, including calcium, and what are the other modes of regulation for motor adaptors? Supporting this less-appreciated role of calcium, a previous study identified Miro as an EF-hand-containing transmembrane protein of the outer mitochondrial membrane that binds kinesin-1 in the presence of calcium and prevents microtubule binding to the motor domain (9). Interestingly, Miro also possesses the atypical Rho GTPase domain; however, the significance of this domain in motor regulation is not fully understood. Furthermore, in the model organism *Ustilago maydis*, dynein-dependent ER motility was found to be reduced in cells with defective import of calcium in the ER, indicating that calcium-dependent regulation of dynein function might be an ancient mode of regulation and hints at the existence of dynein adaptors similar to CRACR2a in other organisms (10). Interestingly, EF-hand motifs are also present in other dynein adaptors such as Rab11FIP3 and Ninein and Ninein-like protein. Given that the list of dynein adaptors is rapidly growing, an important challenge will be to understand how

interactions between dynein, dynactin, and adaptor proteins are regulated to induce specific cellular responses.

Acknowledgments

We want to thank Drs. Steve Caplan (University of Nebraska Medical Center, Omaha, NE) and Anindya Ghosh Roy (National Brain Research Centre, Haryana, India) for constructive comments.

Work in the Mahak Sharma laboratory is supported by a Wellcome Trust/Department

of Biotechnology India Alliance grant (IA/I/12/500523), a Science and Engineering Research Board grant (EMR/2017/002273), and intramural funding support from the Indian Institute of Science Education and Research Mohali.

The authors declare no competing financial interests.

1. Zhang, K., et al. 2017. *Cell*. <https://doi.org/10.1016/j.cell.2017.05.025>
2. McKenney, R.J., et al. 2014. *Science*. <https://doi.org/10.1126/science.1254198>
3. Reck-Peterson, S.L., et al. 2018. *Nat. Rev. Mol. Cell Biol.* <https://doi.org/10.1038/s41580-018-0004-3>
4. Matanis, T., et al. 2002. *Nat. Cell Biol.* <https://doi.org/10.1038/ncb891>
5. Horgan, C.P., et al. 2010. *J. Cell Sci.* <https://doi.org/10.1242/jcs.052670>
6. Guo, X., et al. 2016. *Proc. Natl. Acad. Sci. USA*. <https://doi.org/10.1073/pnas.1601844113>
7. Srikanth, S., et al. 2016. *Sci. Signal.* <https://doi.org/10.1126/scisignal.aac9171>
8. Wang, Y., et al. 2019. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201806097>
9. Wang, X., and T.L. Schwarz. 2009. *Cell*. <https://doi.org/10.1016/j.cell.2008.11.046>
10. Adamiková, L., et al. 2004. *Mol. Biol. Cell*. <https://doi.org/10.1091/mbc.e03-09-0675>