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The final twist in endocytic membrane scission

Many endocytic uptake events depend on the 'pinchase' activity of dynamin. By measuring the orientation of single gold nanorods, a new study reveals that invaginated clathrin-coated endocytic pits undergo a strong rotational twist prior to or concomitant with their detachment.

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Endocytosis is a cellular process by which extracellular materials such as nutrients and signalling molecules are internalized via membrane-bound carriers. A large variety of mechanisms involving¹ or independent of² the conventional clathrin coat contribute to the construction of endocytic pits from which cargo-loaded endocytic carriers detach by membrane scission. For the latter, the large GTPase dynamin has attracted much attention as a 'pinchase' even when other fission mechanisms coexist³. Dynamin structure and function have been studied from model membrane systems and cells in culture to whole organisms, and it has been concluded that dynamin favours the detachment of endocytic carriers by forming a helical assembly around the neck of an invaginated endocytic pit and squeezing it (Fig. 1)⁴. Several models of how this might be achieved have been proposed, of which the two-stage model⁵ and the ratchet/constriction model^{6,7} are the most documented. According to the two-stage model, coordinated GTP hydrolysis across the helix favours a strongly constricted state that then leads to the rupture of the neck and the release of the endocytic carrier. According to the ratchet/constriction model, dynamin functions more like a molecular motor to make adjacent dynamin helices progressively slide over each other, eventually also leading to a sufficiently constricted state to favour fission.

New tools are needed to study predictions from both models in the cellular context at the site and time of scission, which has been achieved in the Article by Cheng et al. in this issue of *Nature Cell Biology*⁸. To dissect the final stages of clathrin-mediated endocytosis in cells in culture, the authors used a newly developed single-particle orientation and rotational tracking technique termed 3D-SPORT (Fig. 1a).

Information on the orientation of anisotropic imaging probes (e.g., fluorescent dipolar emitters or gold nanorods) can be retrieved by different techniques, including polarized fluorescence microscopy or

Fig. 1 | 3D-SPORT on dynamin's function in endocytosis. **a**, Microscopy on a patch of plasma membrane for concomitant analysis of gold nanorod particle orientation and fluorescent light from dynamin and clathrin. **b**, Dynamin-driven super twist in the sequence of events leading to membrane scission. Scission occurs when GTP is hydrolyzed to GDP + Pi. The arrow indicates time (t). **c**, From live cell experiments, the orientation of single gold nanorod particles is measured in function of time, here for the stages (i) and (ii) as indicated in **b** before and after the super twist, respectively. The 3D orientation of the gold nanorod particle is depicted as the polar angle θ and the azimuth angle ϕ .

defocused dark-field imaging as used in the current study⁸. Polarization is a property of light that can be exploited with the purpose of increasing the full scope of information that can be obtained by photonic imaging. Some polarization microscopy developments have focused on encoding information

on orientation and wobbling into the shape of a single molecule's point spread function (PSF)^{9,10}. Other techniques use ratiometric intensity measurements between different polarization projections^{11,12}. Taking advantage of the plasmon-resonance-associated birefringence of gold nanorods

(i.e., the scattering of light that is polarized along their main axis) and a Nomarski-type of differential interference contrast microscope for defocused dark-field imaging, it is possible to retrieve the orientation of these gold nanorods⁸.

The authors of the current study have coupled gold nanorods to a well-established endocytic cargo protein, the iron transporter transferrin⁸. Using 3D-SPORT in living cells, it was possible to track the gold nanorods in 3D with a time resolution of about 20 ms and a spatial precision of around 10 nm and to determine at the same time their orientation changes with a precision of less than 2 degrees (Fig. 1). Furthermore, the authors' 3D-SPORT setup also allows two additional color channels to be collected simultaneously, here for the imaging of dynamin and clathrin in genome-edited SK-MEL-2 melanoma cells. Very clearly, this approach represents a technical tour de force.

In these experiments, the detachment point of the endocytic carrier was deduced from three main criteria: a peak of dynamin accumulation, a sudden movement of the carrier into the cells' interior space, and the gain of active rotational motion resulting from the loss of a rigid link to the plasma membrane. Clathrin stayed for a few seconds on the inward moving carriers before being shed, as expected for canonical clathrin-dependent endocytosis events¹.

The groundbreaking contribution of the current study comes from analysis of the rotational behaviour of the transferrin-coupled gold nanorod in relation to accumulation of clathrin and dynamin and the detachment point. The authors described a succession of stages at which variable degrees of rotation were observed that likely represent the molecular events that occur when the clathrin-coated pit is built (i.e., initial membrane curvature and clathrin recruitment, cargo immobilization, progressive invagination of the nascent pit, and dynamin recruitment leading to the squeezing of the neck region). The most dramatic event was observed around the detachment point: a sharp right-handed super twist movement of the cargo-loaded gold nanorods that preceded membrane fission and endocytic vesicle release (Fig. 1b,c). The average twisting angle (130°) was large, and the duration of the twisting movement was very short (0.28 s). This will be key for the discussion on the implications of the super twist discovery for the mechanism of dynamin function.

The authors next considered an important control experiment. The K44A mutant of dynamin has reduced affinity for GTP, partially inhibiting its GTPase and fission activities¹³. In cells that expressed this mutant, not only was endocytosis blocked as expected, but also strikingly the super twist was absent. It could therefore be concluded that GTP hydrolysis by dynamin is required for the molecular rearrangements that lead to the sudden rotational movement of the super twist in the context of membrane fission and endocytosis.

The most important contribution of the current study is the demonstration that dynamin also produces torque in the cellular context. This had been discovered in vitro⁶, but whether it was also true at sites of endocytosis had remained unexplored. Is the dynamin riddle thereby solved? Not quite yet, as a number of outstanding questions remain. One concerns the model—two-stage or ratchet/constriction—that describes best the molecular mechanism of function of dynamin. Notably, the abruptness of the super twist movement tends to argue in favour of the two-stage model. However, as observed by the authors themselves, possible sub-steps as predicted by the ratchet model might be lost if these were even faster than the time and angular resolution of the current 3D-SPORT setup. Molecular simulations may help to close this gap in future studies. Another important question concerns the structure of the dynamin helices before and after the super twist. Different helical states of dynamin (i.e., non-constricted, constricted, and super-constricted) have been described¹⁴, and the knowledge of those that feed into super twisting and that result from it would likely provide critical clues to the underlying molecular events. Finally, the authors describe the existence of a static phase that precedes the super twist⁸, which points to a specific regulation or external triggering whose molecular nature remains to be unraveled.

Beyond its exciting contribution to the understanding of the molecular mechanism of dynamin function in the cellular context, the current study more generally highlights the power of orientation-sensitive microscopy techniques and the larger contribution that these techniques might make in the field of membrane biology. Other forms of orientation-sensitive microscopy include polarization-based fluorescence imaging

techniques, using polarization splitting or PSF engineering. In principle, these allow enrichment of the information from light microscopy imaging by monitoring how the environment of anisotropic probe-tagged biological macromolecules, notably proteins and lipids, affects their rotational and/or orientation freedom (e.g., when they get trapped into endocytic pits). An implementation of orientation-sensitive microscopy with multi-angle total internal reflection fluorescence microscopy can be designed to increase the axial resolution on fixed samples, or when coupled with single-particle tracking photoactivated localization microscopy to achieve super-resolution orientation imaging on live cells. Orientation studies as presented in the current study could even be extended to tissue imaging using polarized nonlinear microscopy, such as second-harmonic generation, of metal nanostructures¹⁵.

1. Kaksonen, M. & Roux, A. *Nat. Rev. Mol. Cell Biol.* **19**, 313–326 (2018).
2. Johannes, L., Parton, R. G., Bassereau, P. & Mayor, S. *Nat. Rev. Mol. Cell Biol.* **16**, 311–321 (2015).
3. Renard, H.-E., Johannes, L. & Morsomme, P. *Trends Cell Biol.* **28**, 274–286 (2018).
4. Antony, B. et al. *EMBO J.* **35**, 2270–2284 (2016).
5. Schmid, S. L. & Frolov, V. A. *Annu. Rev. Cell Dev. Biol.* **27**, 79–105 (2011).
6. Roux, A., Uyhazi, K., Frost, A. & De Camilli, P. *Nature* **441**, 528–531 (2006).
7. Colom, A., Redondo-Morata, L., Chiaruttini, N., Roux, A. & Scheuring, S. *Proc. Natl Acad. Sci. USA* **114**, 5449–5454 (2017).
8. Cheng, X. et al. *Nat. Cell Biol.* <https://doi.org/10.1038/s41556-021-00713-x> (2021).
9. Curcio, V., Alemán-Castañeda, L. A., Brown, T. G., Brasselet, S. & Alonso, M. A. *Nat. Commun.* **11**, 5307 (2020).
10. Zhang, O., Lu, J., Ding, T. & Lew, M. D. *Appl. Phys. Lett.* **113**, 031103 (2018).
11. Valades Cruz, C. A. et al. *Proc. Natl Acad. Sci. USA* **113**, E820–E828 (2016).
12. Mehta, S. B. et al. *Proc. Natl Acad. Sci. USA* **113**, E6352–E6361 (2016).
13. van der Bliek, A. M. et al. *J. Cell Biol.* **122**, 553–563 (1993).
14. Jimah, J. R. & Hinshaw, J. E. *Trends Cell Biol.* **29**, 257–273 (2019).
15. Balla, N. K. et al. *ACS Photonics* **4**, 292–301 (2017).

Competing interests

The authors declare no competing interests.