Dynamin remodeling of the dynamin helix during membrane constriction

Adai Colom\textsuperscript{a,b}, Lorena Redondo-Morata\textsuperscript{c}, Nicolas Chiaruttini\textsuperscript{d}, Aurélien Roux\textsuperscript{a,b,1}, and Simon Scheuring\textsuperscript{c,d,e,1}

\textsuperscript{a}Department of Biochemistry, University of Geneva, CH-1211 Geneva, Switzerland; \textsuperscript{b}Swiss National Centre for Competence in Research Programme Chemical Biology, CH-1211 Geneva, Switzerland; \textsuperscript{c}Unite 1006, INSERM, Aix-Marseille Université, FR-13009 Marseille, France; \textsuperscript{d}Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY 10065; and \textsuperscript{e}Department of Anesthesiology, Weill Cornell Medicine, New York, NY 10065

Edited by Pietro De Camilli, Howard Hughes Medical Institute, Yale University, New Haven, CT, and approved April 11, 2017 (received for review November 30, 2016)

Dynamin is a dimeric GTPase that assembles into a helix around the neck of endocytic buds. Upon GTP hydrolysis, dynamin breaks these necks, a reaction called membrane fission. Fission requires dynamin to first constrict the membrane. It is unclear, however, how dynamin helix constriction works. Here we undertake a direct high-speed atomic force microscopy imaging analysis to visualize the constriction of single dynamin-coated membrane tubules. We show GTP-induced dynamic rearrangements of the dynamin helix turns: the average distances between turns reduce with GTP hydrolysis. These distances vary, however, over time because helical turns were observed to transiently pair and dissociate. At fission sites, these cycles of association and dissociation were correlated with relative lateral displacement of the turns and constriction. Our findings show relative longitudinal and lateral displacements of helical turns related to constriction. Our work highlights the potential of high-speed atomic force microscopy for the observation of mechanochemical proteins onto membranes during action at almost molecular resolution.

Significance

The GTPase dynamin catalyzes membrane fission and is essential in endocytosis and other events such as organelle division. Dynamin is a unique molecular motor with torsional and contractile abilities. Because these abilities involve a conformational change at the whole-polymer level, standard structural biology tools have not been able to fully unravel the mechanism by which it constricts and twists. Here we used high-speed atomic force microscopy to image the constriction and fission of dynamin-coated tubules with subnanometer and subsecond resolution. Our results provide important findings to establish the contribution of the various constriction mechanisms.

Author contributions: A.C., L.R.-M., A.R., and S.S. designed research; A.C. and L.R.-M. performed research; N.C. contributed new reagents/analytic tools; A.C., L.R.-M., N.C., A.R., and S.S. analyzed data; and A.C., L.R.-M., A.R., and S.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

\textsuperscript{*}To whom correspondence may be addressed. Email: sis2019@med.cornell.edu or aurelien.roux@unige.ch.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1619578114/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1619578114
Results

Although torsion and compaction are not exclusive, because both mechanisms could occur at the same time in constriction, we ought to visualize the global conformational changes of single dynamin-coated membrane tubules with molecular and subsecond resolutions to better understand how dynamin constricts: we adapted in vitro reconstitution assays for high-speed AFM (HS-AFM) (13), which has recently proven powerful for the study of membrane remodeling proteins on mica-supported bilayers (14). We found that the adhesion of the proteins to the mica could impair the dynamin helix conformational change. To overcome this technical limitation, we coated the mica with biotin-lipid bilayers and attached partially (10%) biotinylated dynamin tubules via streptavidin. We reasoned that with one tenth of the dynamins being biotinylated, we would have on average about one functionalized dynamin per helix turn and hence anchorage of the tubules to the support about every 10 turns. This strategy generates links strong enough to avoid displacement of the tubules during HS-AFM scanning but spreads attachment points far enough for providing motional freedom (15) and allowing to observe conformational changes of the dynamin helix (see Materials and Methods for details).

First, experiments were performed with 100% dioleoylphosphatidylserine (DOPS) liposomes (16) mixed with deleted-Proline-Rich-domain (ΔPRD) human dynamin-1 expressed and purified from bacteria (1). We used 100% DOPS because it favored the formation of long dynamin-coated tubules in electron microscopy assays (16, 17). We confirmed that ΔPRD-dynamin bound to DOPS liposomes and deformed them into long membrane tubules decorated by a dynamin helix (Fig. 1 B and C), as previously observed (16, 18). ΔPRD-dynamin was used instead of full-length dynamin because absence of the flexible PRD domain resulted in better resolution in HS-AFM images (Fig. S1 A and B)

The average thickness of these tubules was 63.0 ± 10.4 nm (n = 28) (mean ± SD, throughout the text, unless noted), and the pitch of the striations was 19.2 ± 3.6 nm (n = 141 turns on four tubules; Fig. 1 C). We measured similar values in EM images (diameter, 59.0 ± 4.5 nm; n = 26, pitch, 15.0 ± 4.5 nm, n = 38 tubules), consistent with previous reports (16). It is noteworthy that HS-AFM contours only the protein surface, which in the case of dynamin is composed of helical dimers. Whenever we refer in the text to dimers, we examine the structure and position of these surface exposed domains—helical dimers—and cannot provide information about intramolecular conformational changes.

We then added 10 μL of a 10-mM GTP solution to the 90-μL fluid chamber volume, resulting in 1.1 mM GTP. Right after GTP addition, we often observed tubule constriction (Fig. 1 B–D, Movie S1). Constriction, however, appeared very inhomogeneous, with some parts remaining unconstricted and others narrowed. This constriction was not due to forces applied by the AFM tip onto the tubule, because the same constriction was visible on other tubules when the field of observation was widened (compare Fig. 1 B with Fig. 1 D, Fig. S1 C–H, and Movie S1). As well, the constriction was also observed in the more physiological conditions in which full-length dynamin was used to generate tubules out of liposomes formed of brain extract lipids supplemented with 15% PIP2 (Fig. S1I). The dynamin coat remained mostly attached, and striations were visible during the constriction of the tubules, even though the regularity of the pattern was strongly affected (Fig. 1 C and Fig. S1 D and F).

At some of the most constricted locations, the tubule was virtually
invisible, suggesting that fission may have occurred at these sites (Fig. 1C and Fig. S1, orange arrowheads).

To achieve higher temporal resolution imaging of the constriction, we acquired HS-AFM movies at 0.96 s per frame (Fig. 2 and
Movie S2) and 1.5 s per frame (Fig. 2B and Movie S3). When GTP was added to the observation chamber, a slow constriction of the dynamin-coated tubules was observed (Fig. 2A–C, F, and G and Fig. S2 A–C). Such continuous constriction of tubules was never observed in the absence of GTP (Fig. 2H) or after addition of GDP·AlF₄⁻ (Fig. S3 A–C). A minor, homogeneous constriction was observed in the presence of GMP·PCP (Fig. S3 D–H), consistent with the cryo-EM data that showed a more constricted state when ΔPRD-dynamin was loaded with GMP·PCP (6, 7).

Upon GTP addition, the initial tubule thickness of 60–70 nm reduced to 20–30 nm in the most constricted sites (Fig. 2F and Fig. S2 A–C). Compared with previous estimates of the constriction dynamics of 0.5–1 s (12), the slow constriction dynamics observed in these movies is most probably due to friction with the surface and steric hindrance caused by the streptavidin/biotin bonds because some tubules showed faster constriction upon a single GTP addition (e.g., Fig. 1C). However, these movies have lower resolution, which suggests that these tubules have fewer bonds with the surface, being less constrained to move. However, the continuous, slow constriction observed (Fig. 2A and B and Movies S2 and S3) argues for an active process triggered by multiple cycles of GTP hydrolysis, rather than an abrupt, single-event conformational change.

After the slow progressive constriction of the entire tubule, a more rapid reduction of the tubule thickness at the most constricted locations was observed (see between times 40 and 50 min in Fig. 2F). However, the HS-AFM tip still recorded a height of 3.1 nm, insufficient to visualize the details of the helical reorganization (13, 20).

In some constricted parts of the tubule, the resolution was high enough to resolve the helical turns of dynamin (Fig. 2D and Fig. S2 D and E). Our observations show that fission occurred where the helical turns are the most constricted (Fig. 2D and Fig. S2D). Due to limitations of how far the tip can penetrate between dynamin turns, it is not detectable in these images whether partial disassembly occurred at the fission site or not. It is, however, clear that highly constricted turns are in close vicinity to the fission site and that the depth within the fission site is significantly deeper than the one of the surrounding constricted turns (Fig. 2D, end of kymograph). Thus, fission clearly occurred where the curvature gradient along the tubule axis was highest, as previously proposed (19).

During constriction of the tubule, the helical pattern remained visible most of the time (Fig. 2D and Fig. S2D). Some of the helical turns moved apart, and some seemed to collapse into a single turn and/or split upon GTP addition, which we interpret as pairing and dissociation of neighboring turns (arrows in Fig. 2A and D; see also Fig. 4I). Moreover, the intensity, i.e., the height of the turns, greatly varied with time, as expected during constriction. On the contrary, neither lateral rearrangements nor height variations were observed in the absence of GTP (Fig. 2E) nor in the presence of GMP·PCP or GDP·AlF₄⁻. These variations and rearrangements are unrelated to fluxes occurring in the chamber upon GTP addition because they did not occur upon buffer addition. We quantified these rearrangements: on average, the helix pitch reduced from 19.2 ± 3.6 nm (n = 141) to 15.2 ± 4.9 nm (n = 38) (Fig. 2I), consistent with the helix height profile showing closer turns (Fig. 2I), yet the SD, i.e., the variability of the pitch, increased. Along with this helix shortening, we observed a significant change of turn lateral thickness (see blue arrows in Fig. 2D). This could be due to turn pairing, as described above, or to a change of the angles between turns and the tubule axis: from a sharp distribution around 90°–95° in absence of GTP, the angles spread from 50° to 105° with GTP (Fig. 2K). This change of orientation was highly dynamic (Fig. 24, orange arrow, and Fig. S2E). Altogether, our results show that GTP hydrolysis changes a rather regular helix into a highly dynamic and variable structure on the way to fission, a behavior that could not be pictured by the previous static, averaged structures of crystallographic and cryo-EM data.

The resolution of the images on DOPS tubules was, however, insufficient to visualize the details of the helical reorganization process at the single-protein level, most probably because DOPS tubules have a low rigidity limiting HS-AFM resolution (13, 20). To improve HS-AFM contouring and thus the resolution of the images, we opted for the use of rigid lipid nanorods formed by the spontaneous assembly of galactocerebrosides (21, 22). Galactocerebrosides were supplemented with 5% PIP₂ to mediate dynamin binding to the nanorods (21). Nanorods are rigid and cannot be constricted by dynamin. Indeed, once assembled onto these templates in absence of GTP (Movie S4), substructures of...
tubules (Fig. 2f). In other cases, height profiles showed increasing distances between peaks after GTP addition (Fig. 3d and Movie S6), consistent with a previous report that the helix pitch was larger after GTP hydrolysis on nanorods (21). To explain this variability within our observations, we checked by negative stain EM how dynamin-coated nanorods behaved upon GTP treatment. As previously reported (21), we observed helices with increased pitch distance (Fig. S5 A and B), but we also found compacted helices with a shorter pitch (Fig. S5C), consistent with the pitch reduction observed by HS-AFM (Fig. 3 B and C).

Altogether, these observations show strong dynamics of dynamin helical turns during GTP hydrolysis. Indeed, we were able to observe adjacent turns undergoing dynamic cycles of association/dissociation in presence of GTP (Fig. 4A), but we cannot provide statistics whether more than two neighboring turns can be clustered by such pairing. These observations are consistent with the pairwise collapse and separation of helical turns observed on DOPS tubules (Fig. 2D). This dynamical breathing of the dynamin helix turns suggests that the G–G links can be either tighter, causing the apparent pairing of two adjacent turns, or looser, causing turns to separate, in the presence of GTP (Fig. 4A, white arrows). However, we cannot exclude that these cycles of association/dissociation are not random collisions, because we could not observe molecular links between helical dimers in all experiments. Our results on the dynamic changes observed in the pitch and angle of helical turns show that the constriction observed on membrane tubules is correlated with processive cycles of helical turns pairing and separating, probably consecutive to conformational changes at the level of each dimer. The dynamical breathing of dynamin turns described above is an essential postulate of the torsion model (Fig. 1A): this model implies that dynamins in neighboring turns must dissociate to allow constriction, slide, and reassociate to perform constriction.

However, we also noticed that the distribution of distances between helical dimers along the helical path dramatically changed upon GTP addition (Fig. S2D). This distribution had a single peak centered on 12–14 nm in absence of GTP but changed to a heterogeneous distribution with two apparent peaks in presence of GTP, one around 6–10 nm and the other remaining at 12–14 nm (Fig. S2D). This change of distances could be the result of GTPase domains changing their orientation upon GTP hydrolysis (4). However, this change of distances could also be the result of a helix compaction following a corkscrew intramolecular conformational change (8) (Fig. 1A). We thus looked for further evidence of relative displacement of adjacent helical turns.

Because nanorods do not allow constriction, we looked for evidences of turn relative displacement on DOPS tubules. Although DOPS tubules are softer than nanorods, occasionally, helical dimers were visible [Fig. 2B, fission point 1 (F.P.1), at higher magnification in Fig. 4B] in particular when the tube was already highly constricted and therefore probably more rigid. Close to fission sites, the evenly spaced helical dimers moved with respect to each other in adjacent turns (Fig. 4B): although the topographic heights—interpreted as helical dimers—are basically aligned facing each other at t = 0 min, 3 s (Fig. 4B, red and blue outlines), the same are later (t = 156 s) in a clearly nonaligned zigzag arrangement. Also, the profiles show that these lateral movements are associated with a reduction of the height of the turns and thus with constriction (Fig. 4B, profiles). These results are evidence of the relative displacement of helical dimers from neighboring turns but do not clearly show relative sliding of turns over a distance larger than the size of a helical dimer.

**Discussion**

Our study shows that GTP hydrolysis induces striking changes in the helical structure of assembled dynamin: First, adjacent helix turns can transiently dissociate and reassociate, probably through transient unbinding of G–G links. Second, the helix constricts concomitantly with these molecular rearrangements. However, this constriction is not homogeneous, which may be linked to the difficulty of propagating the constriction along the length of long helices.
helices (12). Third, fission occurs where constriction is the strongest, consistent with previous findings (19). Also, we did not observe any detectable disassembly of the dynamin coat upon GTP hydrolysis, which may question that disassembly is an important step of the fission reaction (23). Our results are thus in support of a model where dynamin constriction requires a dynamic, progressive reorganization of the helical path through several cycles of GTP hydrolysis, rather than an acute, single-step conformational change occurring at the whole-polymer level (23–25). Even though our study of the dynamics of the topographic surface of dynamin tubules does not provide any information about internal rearrangements of the dynamin coat, our results highlight the potential of HS-AFM to visualize highly dynamic and irregular rearrangements of a protein coat onto a membrane.

Materials and Methods

Lipid Suspensions. All lipids were purchased from Avanti Polar Lipids. The galactocerebrosides were purchased from Sigma.

Large Unilamellar Vesicles. Vesicles were prepared using 100% 1,2-dioleoyl-sn-glycero-3-phospho-cytidylate (DOPS) or DOPS:1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl) (Biotinyl Cap PE) 90:10, mol:mol, mixture or 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC). Biotinyl Cap PE 90:10, mol:mol, mixture. Lipids dissolved in chloroform were dried under N₂, followed by 30 min incubation in a vacuum oven at 30 °C or 2 h in a desiccator. Hereafter, lipids were fully rehydrated with GTPase buffer (20 mM Hepes, 150 mM NaCl, 5 mM MgCl₂, pH 7.4) for 10 min at room temperature (RT), obtaining a 2.5 mg/mL lipid solution. Finally, the lipid suspension was vortexed for 10 s and sonicated for 10 min in a bath sonicator. In the end, a tip sonicator (Active Motif) was applied to the solution during 2 s at 60 W and 20 kHz.

Dynamin-Coated Lipid Preparation. For the lipid tubulation with dynamin, 5 μL of large unilamellar vesicle (LUV) suspension was mixed with 2 μL (0.9 mg/mL) of ΔPRD-dynamin (containing 10% of biotinylated ΔPRD-dynamin) and 10 μL of GTPase buffer during 30 min at RT. For the dynamin-coated nanorods, 5 μL of nanorods suspension were used for the reaction instead.

Supported Lipid Bilayers. For mica-supported lipid bilayers (SLBs), LUVs composed of DPPC:Biotinyl Cap PE 90:10, mol:mol, were deposited onto freshly cleaved mica, incubated for 15 min, and rinsed thoroughly with GTPase buffer. For the formation of two-stacked SLBs, the sample was prepared by first depositing DOTAP GUVs on a freshly cleaved mica disk. After the SLB was formed, the sample was carefully rinsed with GTPase buffer. Then, DOPC:lipid 9:1, mol:mol, were deposited forming a double bilayer, the closest to the mica being the DOTAP bilayer and the farthest being the DOPS bilayer. After rinsing with GTPase buffer, ΔPRD-dynamin was added to give a final concentration of 0.22 mg/mL, incubated for 30 min, and then rinsed again with GTPase buffer before imaging.

High-Speed Atomic Force Microscopy Images. An HS-AFM sample scanning (55–NEX (Research Institute of Biomolecule Metrology) (26) setup equipped with short (7 μm long and 2 μm wide) cantilevers with nominal spring constant of 150 pN nm⁻¹, resonance frequency of about 600 kHz, and a quality factor Q = 1.5 in liquid (Nanoworld) was used for movie acquisition. The microscope was operated in amplitude modulation mode, where the cantilever oscillates at a frequency close to its resonance frequency. The phase shift in the oscillation of the cantilever is used to create the phase images, which provide information about the viscoelastic properties of the material. Herein, both topographic and phase images are reported. Either bare mica or mica covered by DPPC:Biotinyl Cap PE, 9:1, mol:mol, SLBs were used as support, the latter followed by the addition of 0.1 μM streptavidin. Streptavidin was incubated for 5 min and rinsed 10 times with GTPase buffer. Finally, the dynamin-DOPS tubule sample was added and incubated for 30 min at RT. During imaging, GTP, GDP, AlF₄⁻, or GMP-PCP solutions were added directly to the HS-AFM fluid cell, if indicated. HS-AFM movies were analyzed in ImageJ, self-written analysis routines, and WSXM 5.0 software (Nanotec (27)).

ACKNOWLEDGMENTS. The authors thank Oliver Daumke, Pierre-Emmanuel Milhiet, and Peter Hinterdorfer for their comments on the manuscript. The S.S. group acknowledges funding support from Agence Nationale de la Recherche (ANR) Grants ANR-Nano ANR-12-BS09-001 and ANR-Biochimie, Biologie Moleculaire et Structurale (BBMS) ANR-12-BSV8-0006-01 and a European Research Council (ERC) Starting (Consolidator) Grant 310080-MEM-STRUCT-AFM. The A.R. group acknowledges funding support from Human Frontier Science Program, Young Investigator Grant RGY0076-2008; the ERC, Starting (Consolidator) Grant 713536-MEMFIS; and the Swiss National Fund for Research, Grants 131003A_130520 and 131003A_149975.