Letter to the Editor

Structural basis for mechanochemical role of *Arabidopsis thaliana* dynamin-related protein in membrane fission

Dear Editor,

Dynamins and dynamin-related proteins (DRPs) constitute a large superfamily of GTPases throughout animal, plant, and bacteria and play essential roles in core cellular processes (Praefcke and McMahon, 2004). Plant specific dynamin-related subfamilies share essential functions with those in mammalian cell, e.g. clarthrin-mediated endocytosis and fission of mitochondria; yet they also play unique functional roles in plant cells (Hong et al., 2003; Chen et al., 2011; Xue et al., 2011) (Supplementary Figure S1). Key features of dynamin members, including large molecular size, high basal GTP hydrolysis, and self-assembly into filamentous helices, distinguish them from other classical signaling and regulatory GTPases (Praefcke and McMahon, 2004). Dynamins are known to play a dual-role in clathrin-mediated endocytosis, in which the basal activity is necessary for early endocytic events and an assembly-stimulated activity is required in later stages of membrane fission (Sever et al., 1999). A mechanochemical model was presented focusing on dynamin in triggering the vesicle scission stimulated by GTP hydrolysis. In such a model, two distinct mechanisms, i.e. 'pinchase' and 'poppase', were proposed based on GTP hydrolysis induced dynamin vesiculation on liposomes (Sweitzer and Hinshaw, 1998). Differences between the two possible mechanisms focus on tightening the vesicle neck by dynamin oligomer to 'pinching off' the vesicle or a length-wise extension of the dynamin super helix to 'popping off'' of the vesicle mechanochemically (Praefcke and McMahon, 2004).

To clarify the assembling process and working mechanism of dynamin members in plant cell cytokinetic processes, we initiate the functional and structural investigation on *Arabidopsis thaliana* dynamin-related protein 1A (*At*DRP1A). GED of dynamin members is known to be directly associated with the GTPase domain (Chappie et al., 2009) and plays a crucial role in either assembly-stimulated or basal GTPase activity. We therefore engineered a 40-kDa *At*DRP1A variant containing the GTPase domain and C-terminal segment of GED (C_{GED}, residues 585–606) fused by a flexible linker (Figure 1A) (named as *At*DRP1A GG hereafter) as suggested in human dynamin (Chappie et al., 2009). The purified *At*DRP1A GG protein maintained a GTPase activity with K_m value of 563 μM and k_{cat} of 0.11 min^{-1} (Supplementary Figure S2), which is in the range of the dynamin family (Praefcke and McMahon, 2004) and suggests that this variant represents the basic catalytic machinery of *At*DRP1A.

Substrate-dependent oligomerization is known to play a central regulatory role in a number of G proteins (Gasper et al., 2009) including the activity of dynamin's GTPase domain (Chappie et al., 2010). *At*DRP1A GG existed in a monomeric form in the presence of either GDP alone or non-hydrolysable GTP analogues as well as in the absence of additional nucleotides (Supplementary Figure S3A), while mainly existed in a dimeric form when it was incubated with GDP supplemented with NaF and $AlCl_3$ (Supplementary Figure S3A). A hexagonal and a monoclinic crystal form were subsequently obtained with *At*DRP1A GG both in dimeric state (Figure 1B). In both crystal forms, GTPase domain presented a canonical structure of GTPase family and two adjacent GTPase cores associated symmetrically with each other (Supplementary Figure S3B). Among the interacting residues, ^{184}NQDLATSD AIK^{194}, named as the '*trans* stabilizing loop' (Chappie et al., 2010), played a major role in *At*DRP1A GG dimerization (Supplementary Figure S3C). Particularly, $D186^A$ interacted with $G65^B$ thus participating in P-loop stabilization. The side chain of D217 and K218 in the conserved G4 (^{211}TKID^{214}) motif contributed to intra-dimer interaction through contacting with the residues in a 'dynamin specific loop' (Chappie et al., 2010). More importantly, the hydrophilic side chain of D217 formed *in trans* interactions with bound GDP molecule as well as G4 motif of the symmetry mate (Supplementary Figure S3D). Although D186A and D217A mutants both forfeit the substrate-dependent dimer formation (Supplementary Figure S4), D186A showed no detectable influence on basal GTPase activity, while D217A significantly reduced GTPase activity (Figure 1C). These observations were consistent with the structural result that D186 is merely involved in protein–protein interaction while D217 also participated in stabilizing GTP molecule. These results reveal that, like in mammalian dynamins (Chappie et al., 2010), oligomerization of *At*DRP1A is essential for its stimulated GTP hydrolysis but not the basal GTPase activity.

Significantly, distinct differences on ligand binding, active site conformations, and orientations of bundle signaling element (BSE) were observed between the two crystal forms. Whereas the GDP molecule was identified in both crystal forms, AlF_4^-, resembling the γ-phosphate group in the GTP hydrolysis transition status, Mg^{2+} and Na^+, which are crucial for GTP hydrolysis, were only observed in the monoclinic crystal form (Figure 1D). As a consequence of 3.6 Å back bond

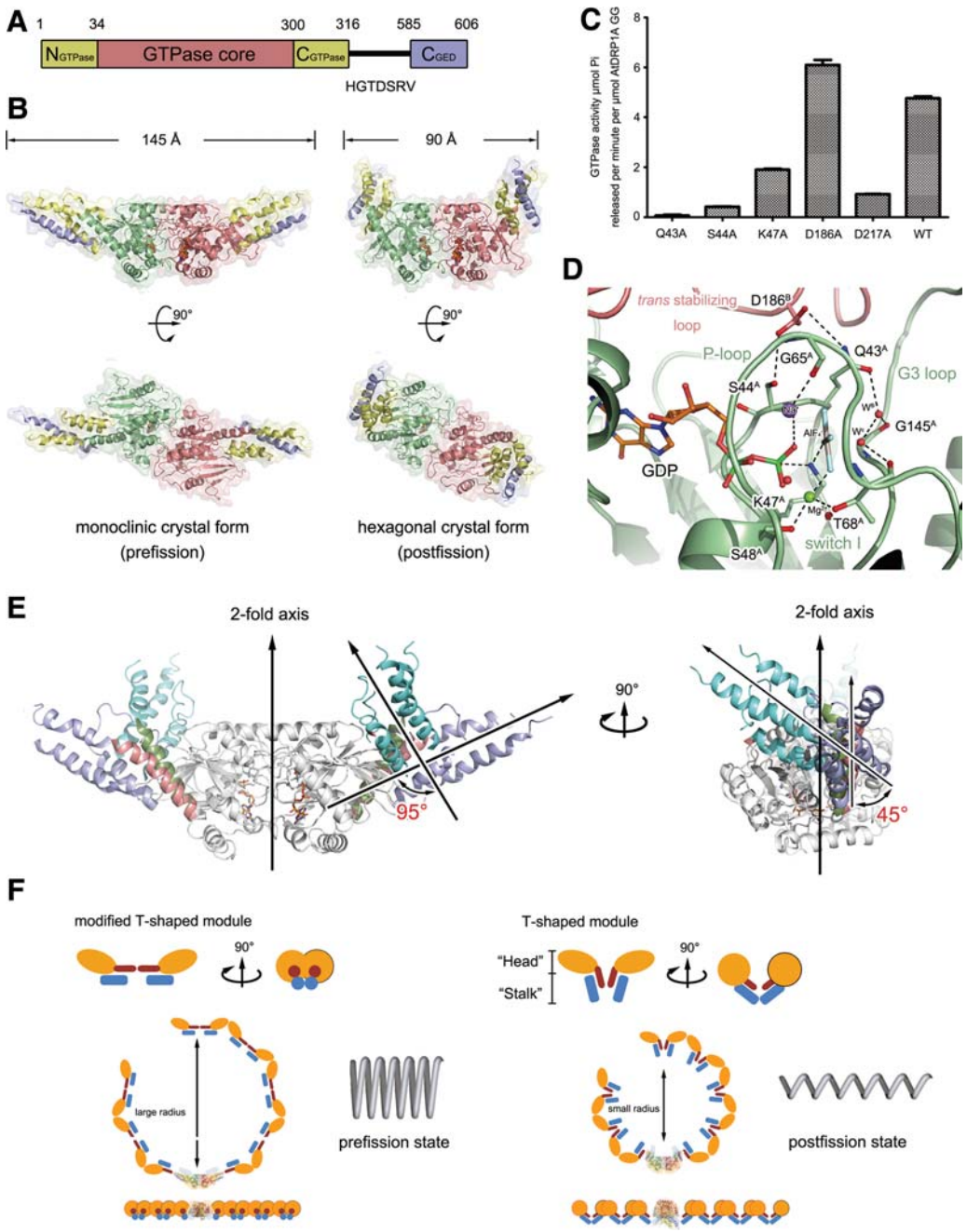

Figure 1 Structure of *At*DRP1A GG support its mechanochemical role in membrane fission. (**A**) Schematic diagram of domain organization of *At*DRP1A GG in primary sequence. The GTPase domain and C-terminal fragment of GED (blue) are connected by an artificial linker (labeled with the linker amino acid sequence). N- (N_{GTPase}) and C- (C_{GTPase}) terminus of the GTPase domain and GTPase core are colored as yellow and red, respectively. (**B**) The structures in monoclinic form (left panel) and hexagonal crystal form (right panel) representing a Pi-bound and Pi-free states. Two protomers of the homodimer are shown in a cartoon representation and colored in green and red, respectively. GED and N- and C-terminus of the GTPase domain which form BSE are colored in blue and yellow, respectively. (**C**) Basal GTPase activities in terms of released Pi of wild-type *At*DRP1A and relevant mutants. (**D**) The catalytic machinery of *At*DRP1A. The catalytic water (W^C), a magnesium ion, and a sodium ion occupying the charge-compensation site are colored in red, green, and purple spheres, respectively. Bound GDP and selected key residues are shown as colored sticks. Functional motifs are labeled. (**E**) Orientation shift of BSEs between prefission and postfission structures. Structures in prefission and postfission state were superposed according to their GTPase core, which is colored white for better presentation. BSEs in prefission and postfission structures are colored as light blue and cyan, whereas red and green for pivotal helix α6. A top view and a side view are shown in left and right panels. The projection angles for two views are labeled out. (**F**) Proposed schematic model based on *At*DRP1A GG structures. A modified T-shaped module of dynamin proposed based on *At*DRP1A GG prefission structure (left panel) and the classical T-shape model (right panel) with two perpendicular views are presented in the corresponding upper panels. In each case, one layer of the spiral ring formed by multiple T-shaped modules and a lengthwise view are shown in the corresponding middle row. One of experimental structure is docked into model for better understanding.

shift in Switch I loop away from the bound GDP molecule in hexagonal crystal form, the hydroxyl atom of T68 was released from a binding with Mg^{2+}, and G63 lost its interaction with β-phosphate of the bound GDP concomitantly (Supplementary Figure S6). Since GDP·AlF_4^- is known to mimic the transition state of GTP hydrolysis and the lack of AlF_4^- is acknowledged to resemble the releasing of Pi, we thus propose that the *At*DRP1A GG structures in monoclinic and hexagonal crystal forms resemble the 'prefission' and 'postfission' states.

N_{GTPase}, C_{GTPase} and C_{GED} form a three-helical BSE to modulate dynamin functions (Chappie et al., 2009; Chappie et al., 2010) Such a BSE is observed in *At*DRP1A GG, which is hinged to GTPase core by a conserved proline residue (Pro303) of classical dynamins with a variable inter-helix elbow angle in pre- and postfission states. The unexpected and distinct orientation shift of BSEs between the pre- and postfission states marked *At*DRP1A uniquely among available structures of dynamins (Figure 1E and Supplementary Figure S8). Indeed, flexibilities between the GTPase core and BSE have been observed in previously reported dynamin members (Reubold et al., 2005; Low and Lowe, 2006; Chappie et al., 2010). However, such a flexibility is usually limited due to stereo-chemical restriction of the conserved proline residue (Chappie et al., 2010). Our observations suggest this pivot proline residue may adopt much larger conformation changes. In postfission state, BSEs (named as BSE^h) tightly interacted with the GTPase core through extensive interactions between residues in N_{GTPase} and residues in the central β-sheet of GTPase core (Supplementary Figure S8C). Consequently, BSE^hs were pulled towards GTPase core and assumed a perpendicular orientation similar to that in human dynamin complexed with GDP·AlF_4^- (Chappie et al., 2010). In contrast, BSEs in the prefission state (named as BSE^m) protruded from the GTPase core in an antiparallel manner. This BSE conformation distinctively differed from the perpendicular orientation in two previously reported crystal forms of human dynamin GG dimer but resembled structures of hGBP1 bound with a non-hydrolysable GTP analog (Prakash et al., 2000) and of the prefusion state of ATL1 (Bian et al., 2011) (Supplementary Figure S7). The strong interaction between BSE^h and GTPase core in the postfission structure was subsequently replaced by a weak interaction mediated by residues of N_{GTPase} and in the loop region connecting β4 and β5 and the C-terminal end of β2 (Supplementary Figure S8).

A tetrameric module of dynamin is acknowledged to function in liposome tubulation through forming a spiral ring, in which the relative orientation of the 'stalk' region to the GTPase domain 'head' determines the radius of the spiral ring formed by dynamin oligomers (Chappie et al., 2009). Our structural and functional results indicate the mechanochemical role played by *At*DRP1A in membrane fission (Figure 1F). First, *At*DRP1A protomers constitute a primary homo-oligomer module; the middle domain and GED assume an extended conformation and wrap around liposome to form a thick helical structure. Upon GTP hydrolysis and Pi releasing, presumably triggered the Switch I movement, 'stalk' regions formed by the middle domain and GED significantly change the conformation and move towards the GTPase core. During this conformational change, a number of new contacts between BSE and GTPase core are established, and two adjacent 'stalks' move from an antiparallel orientation to a perpendicular orientation with a length-wise extend. Consequently, a curvature increase occurs in the dynamin spiral ring surrounding the liposome. On the other hand, the 'stalk' region spatially limits the minimum size between them, and thus the radius of the *At*DRP1A spiral ring may not be able to shrink small enough to directly 'pinch off' the nascent vesicle. Notably, innovative techniques have been introduced to follow the behavior of dynamin *in vitro* and unexpectedly indicate that helix disassembly is also necessary for membrane fission (Bashkirov et al., 2008; Pucadyil and Schmid, 2008), in which the shrink of spiral ring is also necessary for vesicle releasing. The mechanism proposed here for *At*DRP1A could, therefore, also be applied to other membrane fission dynamins in different cellular processes.

However, there remain some gaps in such a model to be filled. For example, it has been reported that an *At*DRP1A/2B complex is pivotal for membrane fission (Fujimoto et al., 2011). The mutagenesis and biochemical results reveal that the wild type *At*DRP2 has extremely lower basal GTPase activity comparing with *At*DRP1A; and, however, the mutations of ^{43}VG^{44} to QS on *At*DRP2 recover its basal GTPase activity (Supplementary Figure S9). Therefore, it is likely that *At*DRP2 will bind with membrane through its PH domain and associate with *At*DRP1A, and *At*DRP1A will subsequently function with conformation change of GED mediated by GTP hydrolysis. Therefore, further structural investigations on full-length plant specific dynamin proteins or their functional complexes in different nucleotide states will be warranted to address this question.

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Liming Yan[1,†], Yuanyuan Ma[2,†], Yuna Sun[3], Jian Gao[1], Xiaoyue Chen[1], Jiewei Liu[1], Congwan Wang[4], Zihe Rao[1,2,3], and Zhiyong Lou[1,*]

[1]Laboratory of Structural Biology and MOE Laboratory of Protein Science, School of Medicine, Tsinghua University, Beijing 100084, China
[2]College of Life Sciences and Tianjin State Laboratory of Protein Science, Nankai University, Tianjin 300071, China
[3]National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Science, Beijing 100101, China
[4]Beijing 107 Middle School, Beijing, China
[†]These authors contributed equally to this work.
[*]Correspondence to: Zhiyong Lou, E-mail: louzy@xtal.tsinghua.edu.cn

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