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Arabidopsis Dynamin-Like Proteins, ADL1C and ADL1E, Play a Critical Role in Mitochondrial Morphogenesis

Jing Bo Jin[P], Hyeunjong Bae[1], Soo Jin Kim[1], Changhyo Go[2], Dae Heon Kim[1], Yong Jik Lee[2], Yu Chung Tse[3], Liwen Jiang[3], Inhwan Hwang[C]

[1]*Center for Plant Intracellular Trafficking, Pohang University of Science and Technology, Pohang 790-784;*
[2]*Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang, 790-784;*
[3]*Department of Biology, Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China*

Dynamin is a high molecular weight GTP-binding protein that is known to be involved in endocytosis in rat brain. Since the discovery of dynamin (now called dynamin I) in rat brain cells, numerous isoforms and homologs have been isolated in a variety of eukaryotic organisms ranging from yeast to humans. A characteristic feature of the dynamin family is the conserved N-terminal GTPase domain. However, the rest of the polypeptide sequence is less well conserved. Althoughdynamin and its related proteins belong to a family of high molecular weight GTPase these proteins are known to be involved in very diverse biological processes. One group of proteins, which includes dynamin I, ADL6, and Vps1p, has been shown to act at various steps of intracellular trafficking, such as endocytosis and vacuolar trafficking. Another group of proteins, which includes DLP1/Drp1, Dnm1p, Mgm1p, and ADL2b, has been shown to be involved in the regulation of mitochondrial morphology. Mutation of these proteins or expression of dominant-negative mutants results in elongation or fusion of mitochondria, indicating that these proteins are involved in mitochondrial fission. In addition, ADL2a has been shown to be targeted to the chloroplast and are thought to be involved in biological processes specific to the plastid. Finally, proteins such as phragmoplastin and ADL1A are thought to play important roles in the formation of the cell division plate in plant. Of the dynamin-like proteins, most studies havebeen done with dynamin I, and the detailed mechanism of action for dynamin I is now well understood at the molecular level. Recently, other members of the dynamin family have also been studied at the molecular level. These studies indicate that dynamin I and related proteins are composed of multiple functional domains. Numerous studies have addressed the biological function of these domains. The proline-rich domain has been shown to interact with the SH3 domain of many proteins and is thought to play a critical role for transmitting the regulatory signals mediated by the SH3-domaincontaining proteins. The pleckstrin homology (PH) domain has been shown to bind to phospholipids and is thought to be responsible for the membrane association of the molecule. The assembly domain is located between the PH domain and the proline-rich domain and is involved in intra- and intermolecular interactions between dynamin molecules, which result in self-assembly of these proteins into a high molecular weight homo-polymeric form. In addition, the assembly domain is proposed to regulate the N-terminal GTPase activity and thereby to function as a GTPase effector domain (GED). Although the PH domain and proline-rich domain appear to be absent from ADL1 and ADL2 of plant cells, DLP1/Drp1 of animal cells, and yeast Dnm1p, these proteins still bind to membranes. For example, ADL2a has been shown to bind to phosphatidylinositol 4-phosphate. Thus, some of these proteins may have a domain for membrane association other than the PH domain.Here, we report the functional characterization of two dynamin homologs in Arabidopsis, Arabidopsis dynamin-like 1C (ADL1C) and Arabidopsis dynamin-like 1E (ADL1E). ADL1C and ADL1E show a high degree of amino acid sequence similarity with members of the dynamin family. However, both proteins lack the C-terminal proline-rich domain and the pleckstrin homology (PH) domain. Expression of the dominant- negative mutant ADL1C[K48E] in protoplasts obtained from leaf cells caused abnormal mitochondrial elongation. Also, a T-DNA insertion mutation at the ADL1E gene caused abnormal mitochondrial elongation which was rescued by transient expression of ADL1C and ADL1E in protoplasts. In immunohistochemistry and in vivo targeting experiments in Arabidopsis protoplasts, ADL1C and ADL1E appeared as numerous speckles and the two proteins colocalized. These speckles were partially colocalized with F1-ATPase:RFP, a mitochondrial marker, and ADL2b localized at the tip of mitochondria. These results suggest that ADL1C and ADL1E may play a critical role in mitochondrial fission in plant cells.