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Molecular cloning of an *Arabidopsis* cDNA encoding a dynamin-like protein that is localized to plastids

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Abstract

Dynamin-related proteins are high molecular weight GTPase proteins found in a variety of eukaryotic cells from yeast to human. They are involved in diverse biological processes that include endocytosis in animal cells and vacuolar protein sorting in yeast. We isolated a new gene, *ADL2*, that encodes a dynamin-like protein in *Arabidopsis*. The *ADL2* cDNA is 2.68 kb in size and has an open reading frame for 809 amino acid residues with a calculated molecular mass of 90 kDa. Sequence analysis of *ADL2* revealed a high degree of amino acid sequence similarity to other members of the dynamin superfamily. Among those members ADL2 was most closely related to Dnm1p of yeast and thus appears to be a member of the Vps1p subfamily. Expression studies showed that the *ADL2* gene is widely expressed in various tissues with highest expression in flower tissues. *In vivo* targeting experiments showed that ADL2:smGFP fusion protein is localized to chloroplasts in soybean photoautroph cells. In addition experiments with deletion constructs revealed that the N-terminal 35 amino acid residues were sufficient to direct the smGFP into chloroplasts in tobacco protoplasts when expressed as a fusion protein.

Introduction

Dynamin is a high-molecular-weight GTPase protein found in the rat brain [34]. Recently many proteins with a high degree of amino acid sequence homology to dynamin have been discovered in various organisms ranging from yeast to man [1, 7, 9, 12, 16, 18, 24, 42, 47, 54, 58]. The primary structures of these proteins are all similar. The N-terminal regions, where the GTP-binding motifs are located, are highly conserved among these proteins. However, the rest of the polypeptide sequences are less well conserved. Also, the biological processes in which these proteins are known to be involved are very diverse [10, 16, 18, 20, 21, 22, 26, 33, 41, 53, 57]. Thus, it appears that the dynamin-like proteins are a superfamily of high molecular weight GTPase proteins. The superfamily can be divided into five subfamilies based on sequence similarities and biological functions: the dynamin, Vps1p, SDL, Mx1, and Mgm1p subfamilies. The dynamin subfamily includes three dynamin isoforms of rat and shibire of *Drosophila* [7, 9, 32, 34, 54]. Much attention has been given to the rat brain isoform, Dynamin I. In numerous studies it has been shown that Dynamin I is composed of multiple functional domains. One of them is the SH3 binding domain which interacts with many proteins [17, 22, 30, 35, 43]. Recently it has been shown that the protein-protein interaction through the SH3 binding domain plays an important role in endocytosis [48]. Another functional domain is the pleckstrin homology (PH) domain at the center of the molecule. It has been suggested that the PH domain is responsible for the membrane association of the molecule by phospholipid-protein interaction [15, 42, 44, 51, 59]. Immunolocalization studies with an-

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number AF012833.

tibodies against Dynamin I have clearly demonstrated that it is present at high concentration at the neck of the invaginated plasma membrane which eventually becomes vesicles [23, 49]. Based on this observation it has been proposed that Dynamin I plays a role in the severing of the neck of the invaginated plasma membrane, thus generating the vesicles during endocytosis. In addition to the two domains discussed so far, Dynamin 1 has a GTPase activity that can be regulated by various means [19, 40, 46, 52] and is critical for endocytosis [10, 22]. Similarly, the *Drosophila* homologue shibire is also thought to be involved in endocytosis [39]. However, other proteins of this subfamily such as the ubiquitously expressed Dynamin II and the testis-specific Dynamin III have been less well characterized with regard to their *in vivo* roles, although Dynamin II has been shown to be localized to the Golgi apparatus [29]. Another well characterized subfamily includes the yeast homologues Vps1p and Dnm1p. Vps1p plays a role in vacuolar protein sorting [56, 57]. It has been proposed that during this process Vps1p participates in the formation of Golgi-derived vesicles which are destined to become vacuoles. Unlike Vps1p, Dnm1p is involved in the endocytotic pathway in yeast [16]. Another dynamin-related protein in yeast is Mgm1p. It forms its own subfamily and is the most distant member of the dynamin superfamily with respect to sequence homology as well as biological function. It plays a role in the maintenance of mitochondrial DNA [20]. Interestingly the molecule has a putative mitochondrial targeting sequence at the N-terminus that is homologous to the bacterial ribonuclease inhibitor, barstar [36]. In addition to the proteins described above, two new genes have recently been identified in plants: *SDL* and *ADL1* [12, 18, 38]. These proteins constitute a separate subfamily. Both of these plant proteins are shown to be associated with membranes and to form a high molecular weight complex *in vivo* [19, 38]. The biological role of the protein encoded by the *ADL1* gene of *Arabidopsis* is thought to be involved in the biogenesis of thylakoid membranes [37] whereas the soybean homologue SDL seems to be involved in the transport of materials to the cell division plate [19]. However, the detailed mechanism by which SDL participates in the transport of materials remains to be elucidated.

In the rat three isoforms of dynamin have been isolated and shown to be differentially expressed. Also, three dynamin-related proteins have been isolated for yeast. Since multiple isoforms of dynamin homologues might also be present in plant cells, we attempted to clone additional genes encoding dynamin-like proteins in *Arabidopsis*. A search through the *Arabidopsis* EST database revealed that there were indeed ESTs that could potentially encode dynamin-like proteins in *Arabidopsis*. Here we report the cloning of an additional cDNA, *ADL2*, that encodes a dynamin-like protein in *Arabidopsis* that is localized to the plastids.

Materials and methods

Screening for cDNAs encoding dynamin-like proteins

The probe for the screening for cDNAs encoding dynamin-like proteins in *Arabidopsis* was prepared by PCR amplification using primers designed based on the nucleotide sequence information deposited in the dbEST database (GenBank accession number, Z29201). The primers were: ADL2-5′: CAGATATCTCGTTTGTTA; ADL2-3′: GGATGTGAAGTGTTTATG. First total RNA was reverse-transcribed to complementary DNA in the following conditions: 2 μg of total RNA, 10 ng of ADL2-3′ primer, 100 units of superscripts reverse transcriptase (BRL, USA), 0.5 mM dNTPs, 10 units of RNase Inhibitor (Promega, USA) in a 20 μl of reaction volume at 37 °C for 1 h. To obtain an *ADL2* probe PCR reaction was performed with 2 μl of the reverse-transcription mixture or 0.1 μg of genomic DNA, 50 ng of each primer in a 100 μl of reaction volume at the following parameters: 30 s denaturation at 94 °C, 30 s annealing at 45 °C, and 30 s elongation at 72 °C for a total of 50 cycles. The PCR products were gel purified and subcloned into pBluescript. Partial nucleotide sequencing was carried out to confirm the PCR products. Subsequently, we screened a lambda cDNA library with the PCR product as the hybridization probe after PCR labeling [14]. Positive plaques were isolated and inserts were excised as pBluescript clones. The insert sizes of the positive clones were compared by digestion with *Eco*RI and *Xho*I and the clone with the largest inserts was selected for further characterization. After confirming the clone by sequencing the 3′ and 5′ ends, serial deletion constructs were generated for full sequencing. The sequencing was done by PCR sequencing using the dideoxy dye terminator according to the manufacturer's protocol (ABI, USA). The nucleotide sequences were read by an automatic sequencing apparatus (ABI, USA).

Northern and Southern blot hybridization

Total RNA was isolated from various tissues by the phenol/LiCl method as described previously [3]. A 20 μg portion of total RNA was separated on a 1.2% formaldehyde-agarose gel and blotted onto a nylon membrane. For Southern blot analysis genomic DNA was isolated and 3 μg of the DNA digested with the appropriate restriction endonucleases. The digested DNA was separated on a 0.8% agarose gel and transferred onto a nylon membrane. The blots were hybridized according to a protocol described previously [8].

Growth of plants

Arabidopsis thaliana (ecotype Columbia) was grown in a greenhouse under the conditions of a 16/8 h light/dark cycle, a temperature of 20 °C, and a relative humidity of 70%. Some plants were grown on MS plates in a growth chamber at 20 °C with a 16/8 h light/dark cycle. Soybean suspension cells (soybean photoautotrophic cells, SB-P) were grown at 25 °C with constant shaking and with a 16/8 h light/dark cycle. Tobacco plants were grown on MS medium supplemented with 2% sucrose in culture bottles at 25 °C with 16/8 h light/dark cycle.

Transformation of fusion constructs into plant cells

Chimeric fusion constructs, *ADL2:smGFP*, *ADL2-BH::smGFP*, and *ADL2EvH:smGFP*, were generated for targeting experiments using standard recombinant technology [3]. A full-length *ADL2* cDNA without the termination codon was prepared by PCR amplification using the *ADL2* cDNA in pBluescript as a template. The primers for the PCR amplification were the T3 primer for pBluescript and a specific primer, ADL2-end (5′-CCTCGAGGAATCGTATCCATTTTG-3′) that eliminated the termination codon. To generate *ADL2BH:smGFP* and *ADL2EvH:smGFP* fusion constructs, the *Bam*HI fragment (200 bp) and *Eco*RV fragment (364 bp) were fused with the coding region of *smGFP* [11]. Transient expression of GFP fusion constructs was carried out after introducing the DNAs into tobacco protoplasts of leaf cells by the PEG-mediated transformation [45] or soybean suspension cells (soybean photoautotrophic cell, SB-P) by the particle bombardment method [4, 50]. Expression of the introduced DNA was examined at various time points after transformation and photographs were taken with a Zeiss Axiophot fluorescence microscope.

Results

Isolation of cDNA clones encoding a dynamin-like protein

In the course of looking at intracellular trafficking in plants, we had cloned and characterized *ADL1* from *Arabidopsis* [38]. To widen our approach we decided to clone additional genes encoding dynamin-like proteins in *Arabidopsis thaliana*. A search through the EST analysis files (University of Minnesota Plant Molecular Informatics Center, USA) resulted in the identification of 12 EST clones with significant sequence homology to dynamin [2]. The analysis of these EST clones suggested that there may be at least 3 additional isoforms of dynamin-like protein genes in *Arabidopsis*. We decided to isolate these genes with the PCR approach. We designed oligonucleotides corresponding to the 5′ and 3′ end of the EST clone, VBVYF04, and used them to generate a hybridization probe by PCR. The RT/PCR amplification from total RNA resulted in a PCR product of 240 bp. Also, PCR amplification using genomic DNA produced a PCR product that was sightly larger than that generated from total RNA (data not shown), indicating that there may be an intron. These PCR products were used to screen an *Arabidopsis* λZAPII cDNA library. We obtained 10 positive cones and pBluescript clones were excised from the λ clones. We named the cDNA clones *ADL2* (*Arabidopsis* dynamin-like 2). The cDNA clone with the largest insert was selected and the nucleotide sequence was determined using a dye terminator sequencing kit. The size of the *ADL2* cDNA was 2.68 kb. The first methionine codon was located at the nucleotide position 95 followed by 2.43 kb of an open reading frame and 161 bp of the 3′-untranslated region.

Sequence analysis of ADL2

The nucleotide and deduced amino acid sequences of the *ADL2* cDNA are shown in Figure 1. *ADL2* has an open reading frame of 2430 bp that encode 809 amino acid residues with a calculated molecular mass of 90 kDa. The size of ADL2 is larger than that of ADL1/aG68 which has 610 amino acid residues with a calculated molecular mass of 68 kDa. The soybean homologue, SDL, also has a similarly smaller size

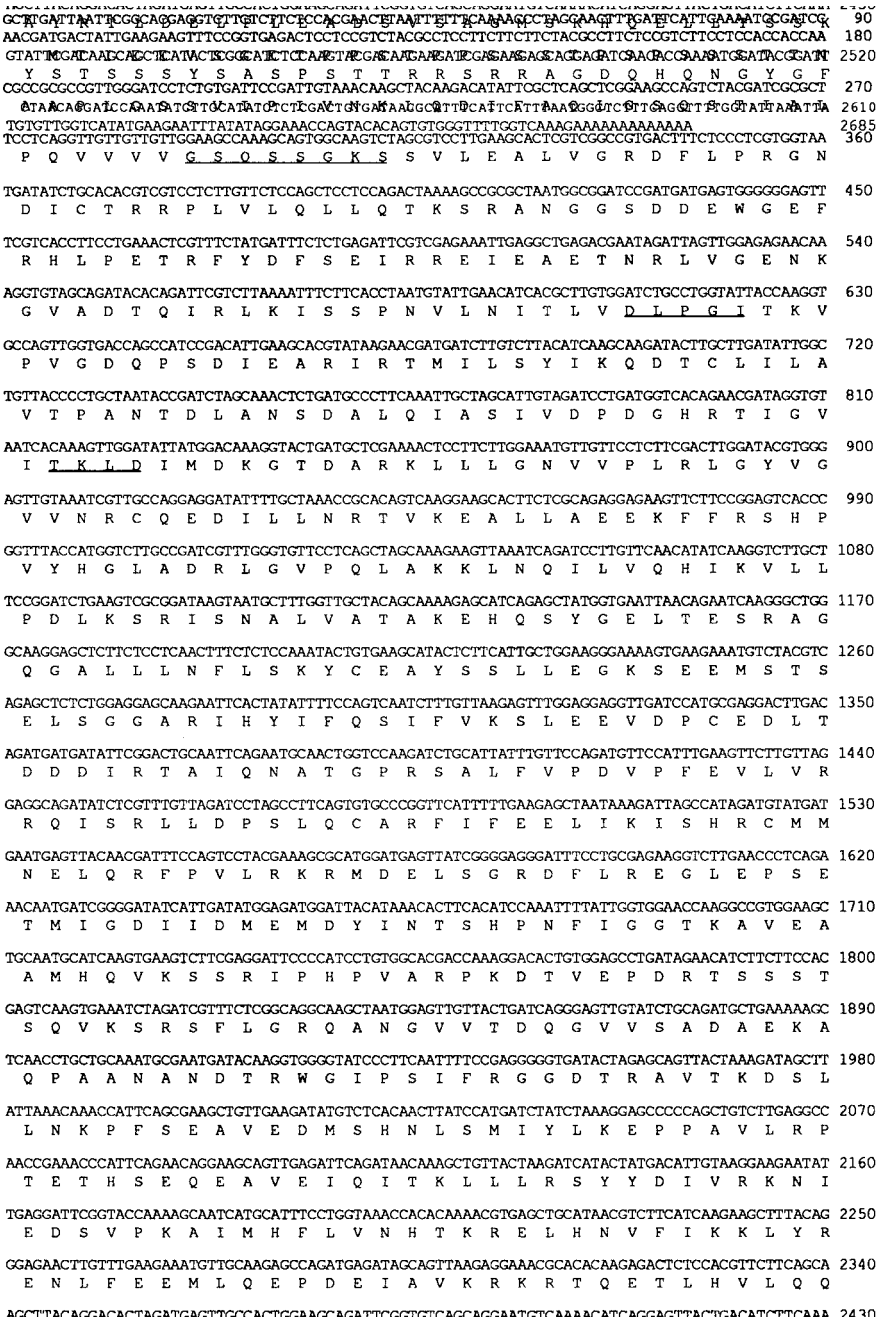

Figure 1. The nucleotide and deduced amino acid sequences of *ADL2*. The putative GTP-binding motifs are underlined.

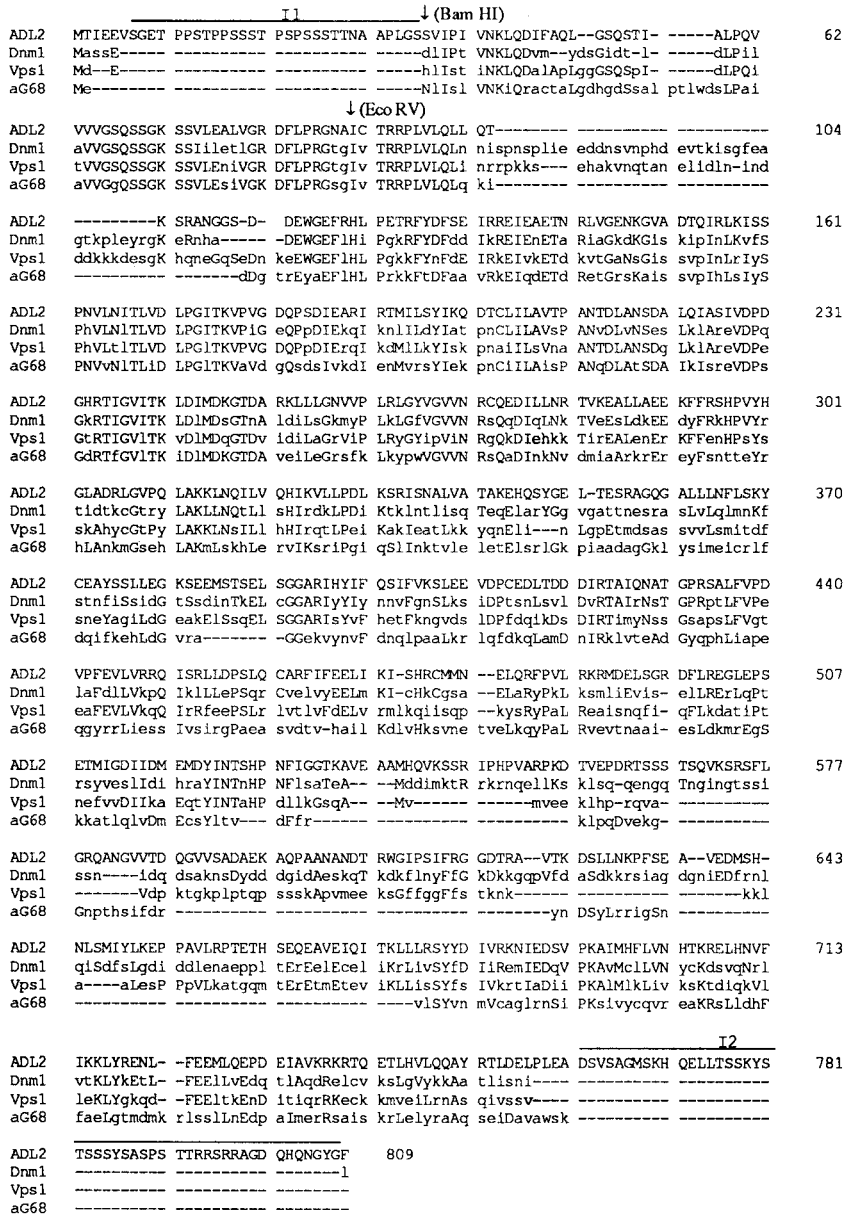

Figure 2. Sequence alignment of ADL2. The amino acid sequence of ADL2 was aligned with the sequences of Dnm1p, Vps1p, and aG68 using the multiple alignment program of DNASIS. Gaps were introduced to maximize identity. Amino acid residues in common with those of ADL2 are indicated by upper case type. I1, and I2 indicate 29 and 45 amino acid insertions, respectively. The *Bam*HI and *Eco*RV sites indicate the restriction sites used to make deletion constructs for the fusion proteins between ADL2 and smGFP as shown in Figure 7.

with 610 amino acid residues [18]. The primary amino acid sequence of ADL2 reveals that it has a structure with similarities to other members of the dynamin superfamily, namely the highly conserved GTP-binding domain in the N-terminal region and the less well conserved region at the C-terminus. However, ADL2 appears not to have the proline-rich SH3 binding domain found in rat Dynamin I and which has been implicated in protein-protein interaction. It is also not clear, whether ADL2 has the other important functional domain, the PH domain which has been shown to interact with phospholipids *in vitro*. In most cases, it is rather difficult to recognize the PH domain in the amino acid sequence by simple inspection of the pri-

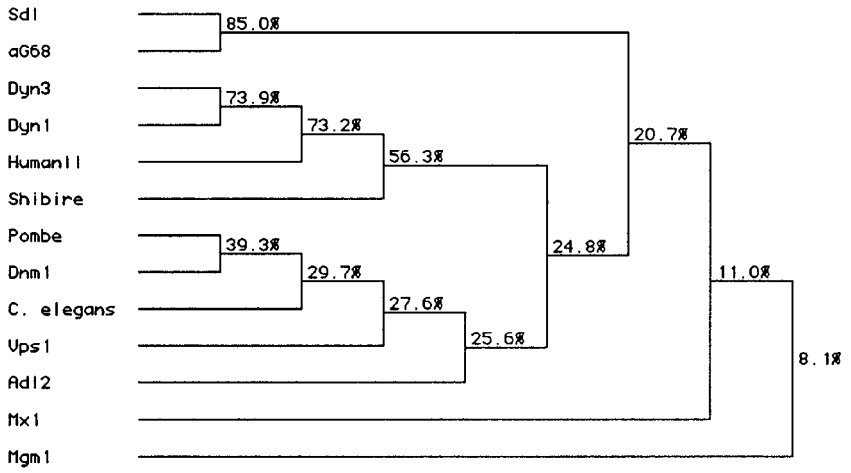

Figure 3. Phylogenetic tree for ADL2. A phylogenetic tree was constructed including 13 amino acid sequences obtained from GenBank and using the multiple alignment program of DNASIS. Sdl (U25547), aG68 (S59558), Dyn1 (p21575), Vps1 (P21576), Dyn3 (Q08877), Human II (L36983), Shibire (P27619), Pombe (Q09748), Dnm1 (P54861), C. elegans (U61944), Mx1(P20591), Mgm1 (S33918)

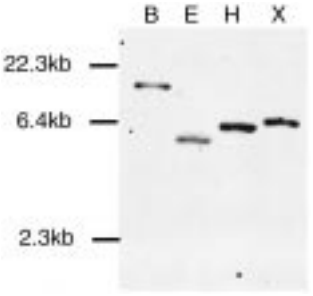

Figure 4. Southern blot analysis of the *ADL2* gene. Genomic DNA was isolated from the Columbia ecotype and 3 μg of the genomic DNA was digested with restriction endonucleases. The digested DNA was separated on a 0.8% agarose gel, blotted onto a nylon membrane, and UV cross-linked. Hybridization was carried out at 65 °C with the randomly labeled cDNA as the hybridization probe. B, *Bam*HI, E, *Eco*RI; H, *Hin*dIII; X, *Xho*I.

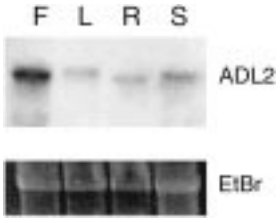

Figure 5. Expression of the *ADL2* gene. A. Total RNA (20 μg) isolated from various tissues was separated on a 1.2% formaldehyde/agarose gel, blotted onto a nylon membrane, and UV cross-linked. Hybridization was at 65 °C overnight to the randomly labeled cDNA as a probe. F, flower; L, leaf; R, root; S, siliques. To check for equal loading, the gel was stained with EtBr before blotting.

mary sequence due to the low degree of amino acid sequence conservation in spite of the fact that the PH domains of various proteins have remarkably similar tertiary structures [15].

Sequence comparisons of ADL2

The deduced amino acid sequence of ADL2 was compared to those deposited in the public databases using the Blastx program provided by the NCBI e-mail server [2]. ADL2 exhibited 38%, 35%, and 26% sequence similarity to Dnm1p [16], Vps1 [41], and aG68/ADL1 [12, 38], respectively. Interestingly ADL2 was more closely related to the yeast proteins Dnm1p and Vps1p than to the plant homologues ADL1/aG68 and SDL. The aligned sequences of ADL2, Dnm1p, Vps1p, and aG68 are shown in Figure 2. The sequence comparison revealed that ADL2 shared the highest degree of amino acid sequence homology in the region containing the GTP-binding motifs. It is known that the homology between the members of the dynamin family is usually limited to the N-terminal region. However, the homology between ADL2 and Dnm1p extends beyond the GTPase domain to the C-terminal region, indicating an even closer relationship between ADL2 and Dnm1p. Close inspection of the sequence alignment revealed additional features. It has been noticed that Vps1p and Dnm1p have 47 amino acid residues inserted between the first and second motif of the GTPase motifs, resulting in an unusually long spacing between the first and the second motif of the GTPase domain [16]. This insertion is absent in ADL2. Instead ADL2 has other

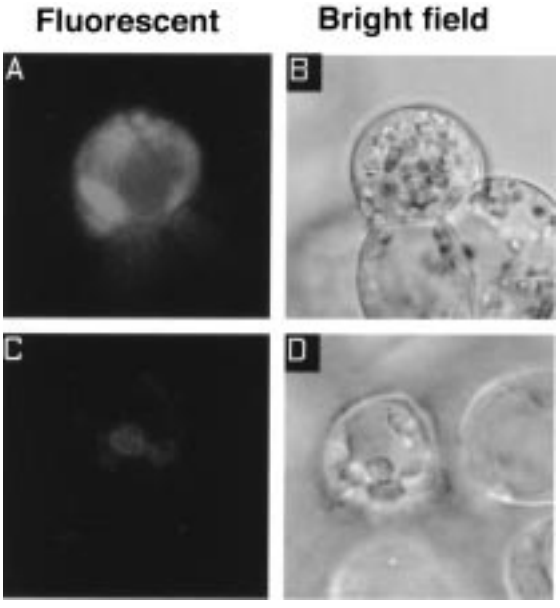

Figure 6. In vivo targeting of *ADL2:smGFP* fusion constructs. The fusion constructs were introduced into SB-P cells by the particle bombardment method and the cells were incubated for 6 h. The soybean cells were examined with a Zeiss fluorescence microscope. Photographs were taken with the Zeiss Axiophot fluorescence microscope. Panels A and B are fluorescent and bright-field images of soybean cells transformed with *ADL2::smGFP*, respectively. Panels C and D are fluorescence and bright field images of soybean cells transformed with *smGFP*, respectively.

insertions in both the N- and C-terminal region. The 29 amino acid insertion (I1 in Figure 2) in the N-terminal region is high of proline and serine content. Sequence comparison revealed that this insertion is present only in ADL2 among the members of the dynamin family. The amino acid sequence composition of the 29 amino acid insertion resembles that of chloroplast transit peptides [5, 27, 55]. It has been shown that the chloroplast transit peptide has a high content of uncharged residues such as serine. Indeed this insertion behaved as a transit peptide for chloroplast targeting (see the targeting of ADL2:smGFP fusion protein below for detail). Also, the C-terminal region of ADL2 has an extension of 45 amino acid residues (I2 in Figure 2) in comparison to Vps1p and Dnm1p. At this moment it remains to be seen whether any functional significance can be attributed to the insertions. To investigate further the relatedness of ADL2 to other members of the dynamin superfamily, we constructed a phylogenetic tree using the multiple alignment program of DNASIS. As shown in figure 3, ADL2 seems to belong to a subfamily made up of the two yeast proteins Vps1p and Dnm1p, homologues of *Caenorhabditis elegans* and *Schizosaccharomyces pombe*. However, it is not clear at this time whether the relatedness of the amino sequences of these polypeptides reflects a functional similarity as well.

Genomic structures of the ADL2 *gene*

To understand the genomic structure of the ADL2 gene, Southern blot analysis was carried out with the cDNA as the hybridization probe. Genomic DNA was isolated from the Columbia ecotype and digested with several restriction endonucleases. Hybridization was carried out under high stringency conditions. As shown in Figure 4, the *ADL2* cDNA appeared as a single band or double bands, suggesting that the *ADL2* gene may be present as a single copy in the genome. When the hybridization was carried out under lower-stringency conditions, there were very weak additional signals (data not shown), suggesting that, if there are any related genes, they may be only distantly related to *ADL2*.

Expression of the ADL2 *gene*

To gain insight into the expression of the *ADL2* gene, we undertook a northern blot analysis using total RNA prepared from various tissues. The whole cDNA was labeled by the random labeling method [14] and used as hybridization probe under the conditions used for the Southern blot analysis. As shown in Figure 5, the *ADL2* gene was expressed in all the tissues we examined. However, there were differences in the level of expression. The highest expression was seen in flower tissue. In a previous study with the *aG68/ADL1* gene, it had been shown that the gene too was expressed in all tissues examined with different levels of expression [12, 38].

Targeting of ADL2::smGFP fusion proteins

The sequence analysis of ADL2 strongly suggested that ADL2 may be localized to plastids. To investigate this possibility further, we tried to localize the protein *in vivo*. A fusion construct was generated between the *ADL2* cDNA and the *smGFP* gene [11] and introduced into soybean suspension cells (SB-P) by the particle bombardment method [50]. Localization of the fusion protein was observed under a fluorescence microscope. As shown in Figure 6, the fusion protein was localized to the plastids of the soybean cells whereas the control smGFP was uniformly distributed in the cytosol. Therefore to further define

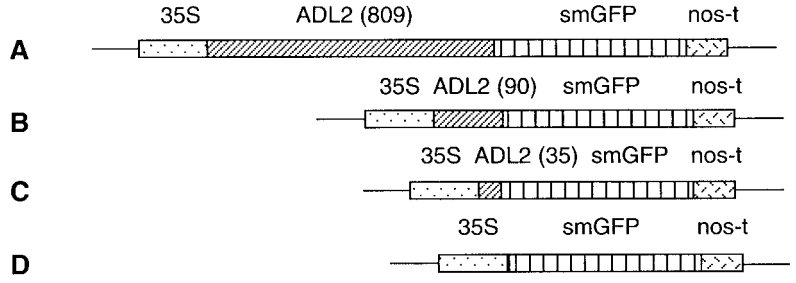

Figure 7. Diagrams of the fusion constructs between *ADL2* and *smGFP*. A, *ADL2:sm:GFP*; B, *ADL2EvH:smGFP*; C, *ADL2BH:smGFP*; D, *smGFP*. 35Spr and nos-t indicate the CaMV 35S promoter and nos terminator, respectively. The restriction sites that were used to construct the deletion mutants are indicated on Figure 2.

the sequence element necessary for the targeting of the protein to plastids deletion constructs of ADL2 were generated and fused to smGFP as shown in Figure 7. The *ADL2BH:smGFP* and *ADL2EvH:smGFP* fusion constructs contained 35 and 90 amino acid residues from the N-terminus, respectively. The fusion constructs were introduced into tobacco leaf cell protoplasts by the PEG-mediated transformation [46]. Localization of the fusion protein was observed with a fluorescence microscope. The introduced fusion genes that were under the control of the CaMV 35S promoter were expressed strongly in the tobacco protoplasts. As shown in Figure 8, all three ADL2:smGFP fusion proteins were clearly localized to the chloroplasts of tobacco protoplasts whereas the control GFP was uniformly distributed. Also, the localization pattern of the ADL2:SMGFP fusion proteins was identical to that of the Cab:smGFP fusion protein (data not shown). To investigate whether the fusion protein is localized inside the chloroplasts, the protoplasts were lysed and treated with proteinase K. The released chloroplasts were remained fluorescent in the presence of proteinase K (data not shown), suggesting that the fusion protein is indeed inside the chloroplasts. Therefore, the *in vivo* targeting experiments with the deletion constructs suggested that ADL2 is targeted into the plastids and that the first 35 amino acid residues are sufficient for the targeting of ADL2 into the plastids.

Discussion

The existence of multiple isoforms of dynamin-like proteins has been known for the rat, for *Drosophila*, and for yeast [7, 16, 29, 32]. However, these multiple isoforms are generated by different mechanisms. In the rat the highly homologous isoforms of dynamin are encoded in three separate genes which are expressed differentially in different tissues [9, 32, 34]. In addition, the primary transcripts of these rat genes are also alternatively processed to yield multiple mRNA species [40]. In *Drosophila*, the *shibire* gene too is alternatively spliced in a tissue-specific manner resulting in multiple isoforms [7]. In yeast there are three genes, *VPS1*, *DNM1*, and *MGM1*, that encode dynamin-like proteins [16, 26, 41]. The amino acid sequences of the dynamin-like proteins show considerable differences and the proteins are involved in different biological processes. In this study we isolated a new gene encoding a dynamin-like protein from *Arabidopsis*. Sequence analysis of ADL2 revealed that it is a new member of the dynamin family. As is the case with other members of the dynamin family, ADL2 has the highly conserved GTP-binding motifs in the N-terminal region, while the rest of the molecule diverges. The ADL2 sequence is most closely related to the yeast Dnm1p with homology extending beyond the highly conserved GTP-binding domains. Thus, ADL2 can be grouped into the Vps1p subfamily. Alignment of the ADL2 sequence with Dnm1p and Vps1p revealed two insertions in both the N- and the C-terminal region. The N-terminal insertion has a relatively high content of proline and serine. In the case of the rat dynamins, the proline rich motifs in the C-terminal region have been shown to be involved in the interaction with a subset of SH3 domains in various proteins [35, 43].

Although Dnm1p and Vps1p are members of the same subfamily, these proteins are involved in different biological processes. Dnm1p is involved in the very early stage of the pheromone-mediated endocytotic pathway in yeast [16], whereas Vps1p is involved in vacuolar protein sorting [41]. It is not clear whether ADL2 shares any functional similarities with these proteins. The *in vivo* targeting experiment with the ADL2:smGFP fusion constructs revealed that

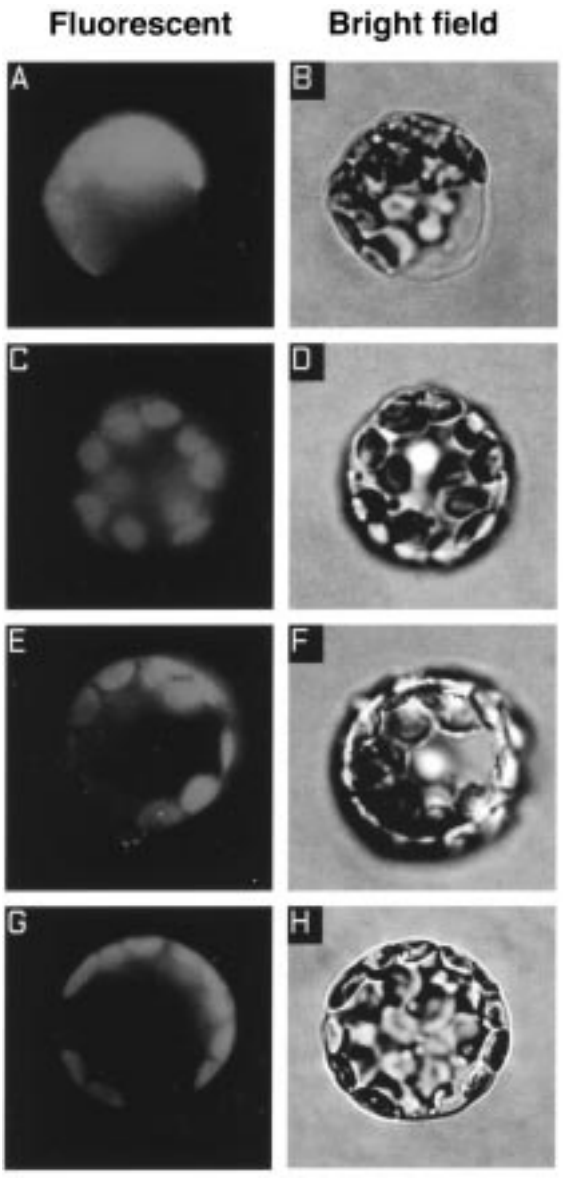

Figure 8. The N-terminal 35 amino acid residues are sufficient for the chloroplast targeting. The fusion constructs were introduced into tobacco leaf protoplasts by the PEG-mediated transformation method and the protoplasts were incubated for 6 to 12 h in the dark. The protoplasts were observed under a fluorescent microscope (Axiophot, Zeiss). Fluorescent (left panels) and bright-field images (right panels) are shown for smGFP (A, B), ADL2:smGFP (C, D), ADL2EvH:smGFP (E, F), ADL2BH:smGFP (G,H).

the fusion proteins were localized at the chloroplasts in tobacco leaf protoplast as well as in soybean suspension culture cells. Interestingly, another dynamin homologue, ADL1 of *Arabidopsis* has shown to be localized to thylakoid membranes of chloroplasts [37]. Dynamin family members are thought to play roles in vesicle formation in a variety of seemingly unrelated processes. The data of the *in vivo* localization experiments thus raise the possibility that ADL2 is involved in vesicle formation in the chloroplasts. In fact it has been demonstrated there is vesicle formation in the chloroplasts. These vesicles were more clearly visible when the leaf tissues of tobacco and pea were incubated at lower temperatures before processing for electron microscope [31]. Also, Hugueney *et al.* [25] have isolated a protein, Pftf, that is involved in the vesicle fusion in the chromoplast of red pepper. It has long been suggested that vesicles could be a means to transport a large amount of lipids from the inner membrane of the chloroplast envelope into the thylakoid membranes during thylakoid membrane biogenesis [6, 13, 31]. Thus, it may be possible that ADL2 is involved in this process. In addition, there are a large number of evidences for the presence of an endomembrane system in the plastids [28]. Therefore, it is equally possible that ADL2 may be involved in the formation of vesicles in this endomembrane system within the plastids.

The transcriptional expression study of the *ADL2* gene revealed that the gene is expressed in all the tissues we examined, but there were differences in the levels of the *ADL2* transcript in the various tissues. The *ADL2* transcript level was highest in flower tissue. However, the flower tissue used for the isolation of total RNA included many different cell types. It will be necessary to define the exact cell type where this gene is most strongly expressed.

Acknowledgements

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References

1. Aebi M, Fah J, Hurt N, Samuel CE, Thomis D, Bazzigher L, Pavlovic J, Haller O, Staeheli P: cDNA structures and regulation of two interferon-induced human Mx proteins. Mol Cell Biol 9: 5062–5072 (1989).
2. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. J Mol Biol 215: 403–410 (1990).
3. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K: Current Protocols in Molecular Biology. Greene Publishing Associates/Wiley-Interscience, New York (1989).
4. Bruce WB, Christensen AH, Klein T, Fromm M, Quail PH: Photoregulation of a phytochrome gene promoter from oat transferred into rice by particle bombardment. Proc Natl Acad Sci USA 86: 9692–9696 (1989).
5. Buvinger WE, Michel H, Bennett J: A truncated analog of a pre-light-harvesting chlorophyll *a/b* protein II transit peptide inhibits protein import into chloroplasts. J Biol Chem 264: 1195–1202 (1989).
6. Carde JP, Joyard J, Douce R: Electron microscope studies of envelop membranes from spinach plastids. Biol Cell 44: 315–324 (1982).
7. Chen MS, Obar RA, Schroeder CC, Austin TW, Poodry CA, Wadsworth SC, Vallee RB: Multiple forms of dynamin are encoded by *shibire*, a *Drosophila* gene involved in endocytosis. Nature 351: 583–586 (1991).
8. Church GM, Gilbert W: Genomic sequencing. Proc Natl Acad Sci USA 81: 1991–1995 (1984).
9. Cook TA, Urrutia R, McNiven MA: Identification of dynamin 2, an isoform ubiquitously expressed in rat tissues. Proc Natl Acad Sci USA 91: 644–648 (1994).
10. Damke H, Baba T, Warnock DE, Schmid SL: Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. J Cell Biol 127: 915–934 (1994).
11. David SJ, Vierstra RD: Soluble derivatives of green fluorescent protein (GFP) for use in *Arabidopsis thaliana.* Weeds World 3: 43–48 (1996).
12. Dombrowski JE, Raikhel NV: Isolation of a cDNA encoding a novel GTP-binding protein of *Arabidopsis thaliana.* Plant Mol Biol 28: 1121–1126 (1995).
13. Douce R, Joyard J: The regulatory role of plastid envelope during development. In: Baker NR, Barber J (eds) Chloroplast Biogenesis, pp. 71–132. Elsevier Science Publishers, Amsterdam (1984).
14. Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132: 6–13 (1983).
15. Ferguson KM, Lemmon MA, Schlessinger J, Sigler PB: Crystal structure at 2.2 Å resolution of the pleckstrin homology domain from human dynamin. Cell 79: 199–209 (1994).
16. Gammie AE, Kurihara LJ, Vallee RB, Rose MD: DNM1, a dynamin-related gene, participates in endosomal trafficking in yeast. J Cell Biol 130: 553–566 (1995).
17. Gout I, Dhand R, Hiles ID, Fry MJ, Panayotou G, Das P, Truong O, Totty NF, Husan J, Booker GW, Campbell ID, Waterfield MD: The GTPase dynamin binds to and is activated by a subset of SH3 domains. Cell 75: 25–36 (1993).
18. Gu X, Verma DPS: Phragmoplastin, a dynamin-like protein associated with cell plate formation in plants. EMBO J 15: 695–704 (1996).
19. Gu X, Verma DP: Dynamics of phragmoplastin in living cells during cell plate formation and uncoupling of cell elongation from the plane of cell division. Plant Cell 9: 157–169 (1997).
20. Guan K, Farh L, Marshall TK, Deschenes RJ: Normal mitochondrial structure and genome maintenance in yeast requires the dynamin-like product of the MGM1 gene. Curr Genet 24: 141–148 (1993).
21. Herskovits JS, Burgess CC, Obar RA, Vallee RB: Effects of mutant rat dynamin on endocytosis. J Cell Biol 122: 565–578 (1993).
22. Herskovits JS, Shpetner HS, Burgess CC, Vallee RB: Microtubules and Src homology 3 domains stimulate the dynamin GTPase via its C-terminal domain. Proc Natl Acad Sci USA 90: 11468–11472 (1993).
23. Hinshaw JE, Schmid SL: Dynamin self-assembles into rings suggesting a mechanism for coated vesicle budding. Nature 374: 190–192 (1995).
24. Horisberger MA, McMaster GK, Zeller H, Wathelet MG, Dellis J, Content J: Cloning and sequence analysis of cDNAs for interferon- and virus-induced human Mx proteins reveal that they contain putative guanine nucleotide-binding sites: functional study of the corresponding gene promoter. J Virol 64: 1171–1181 (1990).
25. Hugueney P, Bouvier F, Badillo A, D'Harlingue A, Kuntz M, Camara B: Identification of a plastid protein involved in vesicle fusion and/or membrane protein translocation. Proc Natl Acad Sci USA 92: 5630–5634 (1995).
26. Jones BA, Fangman WL: Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin. Genes Devel 6: 380–389 (1992).
27. Karlin-Neumann GA, Tobin EM: Transit peptides of nuclear-encoded chloroplast proteins share a common amino acid framework. EMBO J 5: 9–13 (1986).
28. Li X, Henry R, Yuan J, Cline K, Hoffman NE: A chloroplast homologue of the signal recognition particle subunit SRP54 is involved in the posttranslational integration of a protein into thylakoid membranes. Proc Natl Acad Sci USA 92: 3789–3793 (1995).
29. Maier O, Knoblich M, Westermann P: Dynamin II binds to the trans-Golgi network. Biochem Biophys Res Common 223: 229–233 (1996).
30. Miki H, Miura K, Matuoka K, Nakata T, Hirokawa N, Orita S, Kaibuchi K, Takai Y, Takenawa T: Association of Ash/Grb-2 with dynamin through the Src homoglogy 3 domain. J Biol Chem 269: 5489–5492 (1994).
31. Morré DJ, Selldén G, Sundqvist C, Sandelius AS: Stromal low temperature compartment derived from the inner membrane of the chloroplast envelop. Plant Physiol 97: 1558–1564 (1991).
32. Nakata T, Takemura R, Hirokawa N: A novel member of the dynamin family of GTP-binding proteins is expressed specifically in the testis. J Cell Biol 105: 1–5 (1993).
33. Nakayama M, Nagata K, Kato A, Ishihama A: Interferon-inducible mouse Mx1 protein that confers resistance to influenza virus is GTPase. J Biol Chem 266: 21404–21408 (1991).
34. Obar RA, Collins CA, Hammarback JA, Shpetner HS, Vallee RB: Molecular cloning of the microtubule-associated mechanochemical enzyme dynamin reveals homology with a new family of GTP-binding proteins. Nature 347: 256–261 (1990).
35. Okamoto PM, Herskovits JS, Vallee RB: Role of the basic, proline-rich region of dynamin in Src homology 3 domain binding and endocytosis. J Biol Chem 272: 11629–11635 (1997).
36. Pao GM, Saier MH Jr: The N-terminal, putative, mitochondrial targeting domain of the mitochondrial genome maintenance protein (MGM1) in yeast is homologous to the bacterial

ribonuclease inhibitor, barstar. Mol Biol Evol 11: 964–965 (1994).
37. Park JM, Cho JH, Kang SG, Jang HJ, Pih KT, Piao HL, Cho MJ, Hwang I: A dynamin-like protein in Arabidopsis is involved in biogenesis of thylakoid membranes. EMBO J, in press (1998).
38. Park JM, Kang SG, Pih KT, Jang HJ, Piao HL, Yoon HW, Cho MJ, Hwang I: A dynamin-like-protein, ADL1, is present in membranes as a high molecular weight complex in *Arabidopsis thaliana*. Plant Physiol 115: 763–771 (1997).
39. Ramaswami M, Rao S, van der Bliek A, Kelly RB, Krishnan KS: Genetic studies on dynamin function in *Drosophila*. J Neurogenet 9: 73–87 (1993).
40. Robinson PJ, Sontag JM, Liu JP, Fykse EM, Slaughter C, McMahon H, Sudhof TC: Dynamin GTPase regulated by protein kinase C phosphorylation in nerve terminals. Nature 365: 163–166 (1993).
41. Rothman JH, Raymond CK, Giltert T, O'Hara PJ, Stevens TH: A putative GTP binding protein homologous to interferon-inducible Mx proteins performs an essential function in yeast protein sorting. Cell 61: 1063–1074 (1990).
42. Salim K, Bottomley MJ, Querfurth E, Zvelebil MJ, Gout I, Scaife R, Margolis RL, Gigg R, Smith CI, Driscoll PC, Waterfield MD, Panayotou G: Distinct specificity in the recognition of phosphoinositides by the pleckstrin homology domains of dynamin and Bruton's tyrosine kinase. EMBO J 15: 6241–6250 (1996).
43. Seedorf K, Kostka G, Lammers R, Bashkin P, Daly R, Burgess WH, van der Bliek AM, Schlessinger J, Ullrich A: Dynamin binds so SH3 domains of phospholipase C gamma and GRB-2. J Biol Chem 269: 16009–16014 (1994).
44. Shaw G: The pleckstrin homology domain: an intriguing multifunctional protein module. Bioassays 18: 35–46 (1996).
45. Shillito RD, Saul MW: Protoplast isolation and transformation. In: Shaw CH (ed) Plant Molecular Biology: A Practical Approach, pp. 161–186. IRL Press, Oxford, UK (1988).
46. Shpetner HS, Vallee RB: Dynamin is a GTPase stimulated to high levels of activity by microtubules. Nature 355: 733–735 (1992).
47. Shpetner HS, Vallee RB: Identification of dynamin, a novel mechanochemical enzyme that mediates interaction between microtubules. Cell 59: 421–432 (1989).
48. Shupliakov O, Löw P, Grabs D, Gad H, Chen H, David C, Takei K, De Camilli P, Brodin L: Synaptic vesicle endocytosis impaired by disruption of dynamin-SH3 domain interactions. Science 276: 259–263 (1997).
49. Takel K, McPherson PS, Schmid SL, De Camilli P: Tubular membrane invaginations coated by dynamin rings are induced by GTP-gamma S in nerve terminals. Nature 374: 186–190 (1995).
50. Takeuchi Y, Dotson M, Keen NT: Plant transformation: a simple particle bombardment device based on flowing helium. Plant Mol Biol 18: 835–839 (1992).
51. Timm D, Salim K, Gout I, Guruprasad L, Waterfield M, Blundell T: Crystal structure of the pleckstrin homology domain from dynamin. Nat Struct Biol 1: 782–788 (1994).
52. Tuma PL, Stachniak MC, Collins CA: Activation of dynamin GTPase by acidic phospholipids and endogenous rat brain vesicles. J Biol Chem 268: 17240–17246 (1993).
53. Vallee RB, Okamoto PM: The regulation of endocytosis: identifying dynamin's binding patterns. Trends Cell Biol 5: 43–47 (1995).
54. Van der Bliek A, Meyerowitz EM: Dynamin-like protein encoded by the *Drosophila shibire* gene associated with vesicular traffic. Nature 351: 411–414 (1991).
55. Van den Broeck G, Timko MP, Kausch AP, Cashmore AR, Van Montagu M, Herrera-Estrella L: Targeting of a foreign protein to chloroplasts by fusion to the transit peptide from the small subunit of ribulose 1,5-bisphosphate carboxylase. Nature 313: 358–363 (1985).
56. Vater CA, Raymond CK, Ekena K, Howald-Stevenson I, Stevens TH: The VPS1 protein, a homolog of dynamin required for vacuolar protein sorting in *Saccharomyces cerevisiae*, is a GTPase with two functionally separable domains. J Cell Biol 119: 773–786 (1992).
57. Wilsbach K, Payne GS: Vps1p, a member of the dynamin GTPase family, is necessary for Golgi membrane protein retention in *Saccharomyces cerevisiae*. EMBO J 12: 3049–3059 (1993).
58. Yeh E, Driscoll R, Coltrera M, Olins A, Bloom K: A dynamin-like protein encoded by the yeast sporulation gene *SPO15*. Nature 349: 713–714 (1991).
59. Zheng J, Cahill SM, Lemmon MA, Fushman D, Schlessinger J, Cowburn D: Identification of the binding site for acidic phospholipids on the pH domain of dynamin: implications for stimulation of GTPase activity. J Mol Biol 255: 14–21 (1996).