Dnm1p/Vps1p-like protein (DVLP) is a mammalian member of the dynamin GTPase family, which is classified into subfamilies on the basis of the structural similarity. Mammalian dynamins constitute the dynamin subfamily. DVLP belongs to the Vps1 subfamily, which also includes yeast Vps1p and Dnm1p. Typical structural features that discriminate between members of the Vps1 and dynamin subfamilies are that the former lacks the pleckstrin homology and Pro-rich domains. Dynamin exists as tetramers under physiological salt conditions, whereas under low salt conditions, it can polymerize into spirals that resemble the collar structures seen at the necks of constricted coated pits. In this study, we found that DVLP is also oligomeric, probably tetrameric, under physiological salt conditions and forms sedimentable large aggregates under low salt conditions. The data indicate that neither the pleckstrin homology nor Pro-rich domain is required for the self-assembly. Analyses using the two-hybrid system and co-immunoprecipitation show that the N-terminal region containing the GTPase domain and a domain (DVH1) conserved across members of the dynamin and Vps1 subfamilies, can interact with the C-terminal region containing another conserved domain (DVH2). The data on the interdomain interaction of DVLP is compatible with the previous reports on the interdomain interaction of dynamin. Thus, the self-assembly mechanism of DVLP appears to resemble that of dynamin, suggesting that DVLP may also be involved in the formation of transport vesicles.

Dynamin is a high molecular weight GTP-binding protein with an intrinsic GTPase activity. Many proteins that belong to the dynamin family have been identified in a variety of organisms ranging from yeasts to mammals. These proteins have a highly conserved N-terminal GTPase domain of ~300 amino acids (for reviews, see Refs. 1–4). This family is divided into subfamilies on the basis of the structural similarity (2). The dynamin subfamily consists of mammalian dynamins I, II, and III, and the Drosophila shibire gene product. A second subfamily consists of Saccharomyces cerevisiae Vps1p and Dnm1p and a recently identified mammalian member designated DVLP\(^1\) (5), dymple (6), DLP1 (dynamin-like protein 1) (7), or DRP1 (dynamin-related protein 1) (8). Typical structural features that discriminate between members of the Vps1 and dynamin subfamilies are that the former lacks the pleckstrin homology and Pro-rich domains, which are involved in interactions with phospholipids and proteins containing the SH3 domain, respectively. Two plant homologs, phragmoplastin and aG68/ADL1, are grouped into another subfamily. Interferon-inducible Mx proteins and yeast Mgm1p constitute the most diverged subfamily.

Members of the dynamin and Vps1 subfamilies have been implicated in intracellular vesicular transport. Analyses using cells transfected with dynamin mutants and using Drosophila shibire mutants demonstrated that dynamins are responsible for budding of endocytic vesicles from the plasma membrane (9–12). It has also been reported that dynamin II is involved in transport from the trans-Golgi network (13). Morphological analysis and in vitro experiments showed that dynamin self-assembles into rings and/or spirals around the necks of invaginated clathrin-coated pits, thereby suggesting a model that GTP hydrolysis of dynamin pinches off the budding vesicles (14, 15). On the other hand, Vps1p was identified as one of the gene products required for vacuolar protein sorting in yeast (16), and Dnm1p was then isolated as a homolog of Vps1p and may participate in an endocytic process (17). Recently, the mammalian protein most homologous to Vps1p and Dnm1p not only in the primary structure but also in the domain organization has been identified by us and others and designated DVLP (Dnm1p/Vps1p-like protein) (5), dymple (6), DLP1 (7), or DRP1 (8). The most abundant form of DVLP consists of 736 amino acids, but some splicing variants are also present (6, 7). Indirect immunofluorescence microscopy revealed that DVLP is localized to a punctate cytoplasmic structures around the nucleus (5–8). Yo et al. (7) showed by immunoelectron microscopy that DLPL/DVLP-positive structures coalign with microtubules and tubules of the endoplasmic reticulum and proposed that DLPL/DVLP may participate in the formation of nascent secretory vesicles from the endoplasmic reticulum. Velocity sedimentation and gel filtration analyses and cross-linking experiments indicated that dynamin exists as homooligomers, mainly tetramers, under physiological salt conditions (15, 18–21). Under low salt conditions, however, it co-assembles into polymers to form rings and/or spirals (15, 20, 21). During the course of our experiments, we noticed a possibility that DVLP is also oligomeric. We therefore analyzed this phenomenon in detail.

\(^1\) The abbreviations used are: DVLP, Dnm1p/Vps1p-like protein; pleckstrin homology; HA, hemagglutinin; DTSP, dithiothreitol; Gal, beta-galactosidase; DVH, dynamin/Vps1p homology; FL, full-length.
EXPERIMENTAL PROCEDURES

Plasmid Construction—Construction of the expression vector for human DVLP with an N-terminal hemagglutinin (HA) epitope sequence (pcDNA3-HAN-DVLP) was described previously (5). Expression vectors for Myc-tagged DVLP (pcDNA3-Myc-DVLP) was constructed by replacing the fragment encoding the HA epitope of pcDNA3-HAN-DVLP with a double-stranded oligonucleotide for the Myc epitope. A vector for Myc-N- and C-terminal tagged DVLP (pcDNA3-MycN-DVLP; pcDNA3-MycC-DVLP) was constructed by replacing the entire DVLP portion of pcDNA3-MycN-DVLP with a MscI-NoRI fragment of the DVLP cDNA. For the use in the two-hybrid analysis, bait and prey vectors for DVLP were constructed by ligation of a DNA fragment for DVLP from pcDNA3-HAN-DVLP into pGBT9 and pGAD10 (CLONTECH), respectively. Bait and prey vectors for deletion mutants (Fig. 1), except for the prey vector of DVLP(580–736) and DVLP(490–736) were constructed by utilizing the endothelialization sites of the DVLP cDNA; the DNA fragments of DVLP(580–736) and DVLP(490–634) were prepared by a polymerase chain reaction-based strategy.

Antibodies—Sources of antibodies used were as follows: monoclonal rat (3F10) and mouse (12CA5) anti-HA antibodies, Boehringer Mannheim; monoclonal mouse anti-Myc antibody (9E10), Berkeley Antibody Co.; peroxidase-conjugated anti-mouse IgG, Amersham Pharmacia Biotech; other secondary antibodies, Jackson ImmunoResearch Laboratories.

Indirect Immunofluorescence Analysis—A Clone 9 rat hepatocyte cell line stably expressing HA-tagged DVLP (Clone 9/DVLP-HA) was established by selection of clonal cell lines transfected with pcDNA3-HAN-DVLP in the presence of 800 μg/ml Geneticin (Wako Pure Chemicals). For immunofluorescence analyses, Clone 9 cells grown in wells of eight-well Lab-Tek-II chamber slides (Nunc) were transfected with pcDNA3-MycN-DVLP(278–736) alone or in combination with pcDNA3-HAN-DVLP using a TransIT-LT1 transfection reagent (PanVera Corp.), cultured for 20 h, and processed for indirect immunofluorescence analysis as described previously (5, 22). Briefly, cells fixed and permeabilized with methanol at −20 °C for 5 min or those with 4% formaldehyde and permeabilized with 50 μg/ml digitonin for 5 min at 4 °C were incubated with anti-HA and/or anti-Myc antibodies. The cells were then incubated with FITC-conjugated and/or Cy3-conjugated secondary antibodies and observed with a laser-scanning confocal microscope (TCS-NT, Leica Lasertechnik).

Preparation of Cytool, Gel Filtration, and Western Blot Analysis—Clone 9/DVLP-HA cells were homogenized in two volumes of HCB150 (20 mM HEPES-KOH, pH 7.2, 2 mM EGTA, 1 mM MgCl2, 1 mM dithiothreitol, 150 mM NaCl) by 5 sets of 20 strokes with a Dounce homogenizer. The homogenate was centrifuged at 200,000 × g for 60 min in a Beckman TLA 100.2 rotor, and the supernatant was used as a cytosol fraction. The cytosol fraction containing 50 μg of protein was applied to a Superdex 200 column (1.0 cm; Amersham Pharmacia Biotech) equilibrated with HCB150 and eluted at a flow rate of 0.4 ml/min. Fractions of 0.2 ml that were collected were precipitated with cold acetone, dissolved and boiled in SDS-polyacrylamide gel electrophoresis sample buffer, electrophoresed on a 7.5% SDS-polyacrylamide gel under reducing conditions, and electroblotted onto an Immobilon-P membrane (Millipore). The blot was incubated sequentially with anti-HA antibody (3F10) and peroxidase-labeled anti-rat IgG, and detected using a Renaissance Chemiluminescence reagent (Pierce). The reaction product was immunoprecipitated with anti-HA antibody (12CA5) and protein A-Sepharose, electrophoresed on SDS-polyacrylamide gel electrophoresis and Western blot analysis using the monoclonal anti-Myc antibody.

RESULTS

Evidence for Intermolecular Interaction of DVLP in Cells—By indirect immunofluorescence analysis of Clone 9 cells transiently transfected with HA-tagged DVLP, we found that it associated with punctate cytoplasmic structures around the nucleus (6). There was a possibility, however, that the staining pattern was caused by overexpression. We therefore established cell lines stably expressing HA-tagged DVLP at a moderate level. Fig. 2A shows a typical staining pattern for DVLP in one (Clone 9/DVLP-HA) of the established cell lines. As in transiently transfected cells, DVLP was localized to punctate cytoplasmic structures in these stable transfecants. On the other hand, Kamimoto et al. (6) have reported that a dmyc/DVLP mutant lacking the N-terminal GTPase domain forms large cytoplasmic aggregates. In an attempt to reproduce their data, we made a similar construct, DVLP(278–736) along with the HA-tagged DVLP(FL) construct along with the HA-tagged DVLP(FL) construct in Clone 9 cells. As shown in Fig. 2B, we could reproduce the data of Kamimoto et al. (6); the N-terminal deletion mutant was localized to large cytoplasmic aggregates.

During the course of this experiment, we met with unexpected findings. In an attempt to discriminate unequivocally the localization of DVLP(278–736) from that of full-length DVLP (DVLP(FL)), we transfected the Myc-tagged DVLP(278–736) construct along with the HA-tagged DVLP(FL) construct into Clone 9 cells and double-stained with anti-HA and anti-Myc antibodies. We found that in some populations of the cells with a relatively low expression level of DVLP(278–736), the staining for the deletion mutant was superimposed on the punctate cytoplasmic staining for DVLP(FL). The similarity that DVLP may exist as oligomers/ multimers intracellularly.

DVLP Is a Tetramer under Physiological Salt Conditions—To confirm the above speculation, a cytosolic extract was prepared from Clone 9/DVLP-HA cells using buffer containing a physiological concentration of NaCl (HCB150) and subjected to gel filtration on a Superdex-200 column, and the fractions were based prey vector and plated on a medium lacking Try, Leu, and His and containing 10–25 mM 3-aminotriazole (Wako Pure Chemicals). After 5–7 days of incubation, colonies were tested for β-galactosidase (β-Gal) activity by use of replica filter assay. β-Gal activity was also measured by a liquid culture assay using o-nitrophenylgalactoside as a substrate (23).

Co-immunoprecipitation Analysis—Human embryonic kidney 293 cells transiently transfected with either pcDNA3-HAN-DVLP or pcDNA3-Myc-DVLP, or both were washed twice with ice-cold phosphate-buffered saline and lysed in ice-cold cell lysis buffer (50 mM Tris-Cl, pH 7.5, 1% Triton X-100, 2.5 mM EDTA, 0.25 mM NaCl containing CompleteTM protease inhibitor mixture (Boehringer Mannheim)). The lysates were cleared by centrifugation at 12,000 rpm for 10 min in a microcentrifuge. A half of the lysates containing ∼20 μg of protein were directly subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis using anti-HA (3F10) or anti-Myc (9E10) antibody. The other half was immunoprecipitated with anti-HA antibody (12CA5 and protein A-Sepharose and subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis using the monoclonal anti-Myc antibody.

In Vitro Transcription/Translation and Immunoprecipitation Analysis—Either pcDNA3-HAN-DVLP(1–489) alone, pcDNA3-Myc-DVLP(490–736) alone, or both was subjected to in vitro transcription/translation in the presence of an EXPRESS protein labeling mixture (NEB Life Science Products) using a TnT T7 transcription/translation system (Promega Corp.). The reaction product was immunoprecipitated with anti-HA antibody (12CA5) and protein A-Sepharose, electrophoresed on SDS-polyacrylamide gel electrophoresis and analyzed using a BAS2000 imaging analyzer (Fuji Film Co.).
analyzed by Western blotting with anti-HA antibody. As shown in Fig. 3, DVLP-HA eluted from the gel with an apparent molecular mass of \( \sim 320 \) kDa. Because the molecular mass of DVLP-HA monomer is \( \sim 80 \) kDa (Fig. 3; also see Figs. 4 and 7), the species of \( \sim 320 \) kDa is probably a tetramer.

To corroborate the gel filtration data, we then performed cross-linking studies. Clone 9/DVLP-HA cell lysates were treated with a cross-linker, DTSP, in HCB150, and the extent of cross-linking was monitored on Western blots. As shown in Fig. 4, a molecular species of \( \sim 300 \) kDa appeared in a time-dependent manner, with disappearance of the \( \sim 80 \)-kDa band. It is also noteworthy that no dimer/trimer intermediates of DVLP were detected during the incubation, in contrast to cross-linking studies of dynamin (18, 19), in which not only a tetramer but also dynamin species corresponding to a dimer and a trimer were observed. Collectively, these data indicate that DVLP is probably tetrameric in cells.

**DVLP Aggregates under Low Salt Conditions**—Dynamin has been reported to self-assemble into rings and/or spirals under low salt conditions (15, 20, 21). The dynamin assembly can be quantified by a sedimentation assay in which polymerized assembled dynamin appears in the pellet after high speed centrifugation. To examine whether DVLP also polymerizes under low salt conditions, the Clone 9/DVLP-HA cytosol in HCB150 was dialyzed against lower salt buffers and subjected to ultracentrifugation, and the resultant supernatants and pellets were analyzed by Western blotting. As shown in Fig. 5, in higher salt buffers (HCB100 and HCB150), the largest fraction of DVLP remained in the supernatant, whereas in lower salt buffers (HCB0 and HCB50), a significant fraction sedimented. Densitometric analysis revealed that in HCB100 and HCB150 only \( \sim 10 \) and \( \sim 5\% \), respectively, of DVLP sedimented, whereas in HCB0 and HCB50, \( \sim 80 \) and \( \sim 40\% \), respectively, of DVLP assembled into sedimentable complexes.
Intermolecular Interaction of DVLP Molecules—The experiments thus far suggest that, like dynamin, DVLP may exist as tetramers under physiological salt conditions and assemble into sedimentable complexes under low salt conditions. However, it was possible that such complexes might be formed by the aid of an unknown adaptor protein(s), because we used crude cytosolic extracts in the above experiments. To demonstrate the direct intermolecular interaction, we then used the yeast two-hybrid system. When bait and prey vectors fused to DVLP(FL) were co-transformed into reporter yeast cells, the co-transformant grew well on a plate lacking His (Fig. 6A) and showed a high level of β-Gal activity (Fig. 6B). Co-transformation of the DVLP(FL) bait vector with either an empty prey vector (Fig. 6, A and B) or a prey vector fused to an unrelated protein (lamin; not shown) did not result in colony formation on the His-free plate nor show a significant β-Gal activity. The data therefore indicate a specific intermolecular interaction of DVLP.

The above data demonstrated the intermolecular interaction of DVLP in vitro and in the heterologous yeast expression system. To demonstrate such an interaction in cells, we co-transfected cells with vectors for HA- and Myc-tagged DVLP(FL) and examined whether anti-HA antibody was able to co-immunoprecipitate Myc-tagged DVLP from lysate of the co-transfectant. Fig. 7A shows the expression of both HA- and Myc-tagged DVLP in the co-transfectant. When the lysate of the co-transfectant was immunoprecipitated with anti-HA antibody and then subjected to Western blotting with anti-Myc antibody, a specific band of Myc-DVLP in the co-transfectant was detected (Fig. 7B), demonstrating the intermolecular interaction of DVLP in cells.

Interaction between Domains of DVLP—To delineate domains of DVLP responsible for the intermolecular interaction, various deletion mutants were constructed and subjected to the two-hybrid analysis. We anticipated that a C-terminal domain, named dynamin/Vps1p homology 2 (DVH2), could be responsible for such an interaction, because this domain is significantly conserved in the dynamin and Vps1 subfamilies and has the potential to form a coiled-coil structure (1, 5), which has been shown to be involved in homo- and hetero-oligomeric protein-protein interactions. Therefore, we first constructed bait and prey vectors for DVLP(490–736), which includes the DVH2 domain (Fig. 1), and examined whether the homotypic interaction of the coiled-coil domain of DVLP occurred. However, we failed to detect such an interaction (Fig. 6). In contrast, when the prey vector for DVLP(490–736) was co-transformed with a bait vector for the deleted part, namely DVLP(1–489), a significant interaction was observed; the co-transformant grew well on the His-free plate, and its β-Gal activity approximated that of the DVLP(FL)-DVLP(FL) co-transformant (Fig. 6). A similar result was obtained using a combination of a bait vector for DVLP(490–736) and a prey vector for DVLP(1–489) (data not shown). A homotypic interaction of DVLP(1–489) was not detected (Fig. 6). To corroborate the two-hybrid results, we examined whether in vitro translated HA-tagged DVLP(1–489) and Myc-tagged DVLP(490–736) can be co-immunoprecipitated. As shown in Fig. 8, the anti-HA antibody precipitated not only HA-tagged DVLP(1–489) but also Myc-tagged DVLP(1–489). Although the origin of a band between those of HA-DVLP(490–736) and Myc-DVLP(1–489) is not clear, it may be a degradation product or a not fully translated product of DVLP(1–489) and DVLP(490–736). Although the origin of a band between those of HA-DVLP(1–489) and Myc-DVLP(490–736) is not clear, it may be a degradation product or a not fully translated product of HA-DVLP(1–489), because the band was also detected in the lane in which the HA-DVLP(1–489) construct was subjected to in vitro translation and immunoprecipitation with anti-HA antibody.

DVLP(1–489) covers the N-terminal GTPase domain and another conserved domain, named DVH1. In order to determine which region of DVLP(1–489) is responsible for the interaction with DVLP(490–736), we successively truncated the DVLP(1–489) construct from its C terminus (Fig. 1) and examined whether the truncation mutant was able to interact with

---

**Fig. 5. Sedimentation assay.** Cytosol of Clone 9/DVLP-HA cells in HCB150 was dialyzed against HCB0, HCB50, or HCB100 and centrifuged at 200,000 × g for 15 min. The pellet (P) and supernatant (S) fractions were analyzed by Western blotting with anti-HA antibody.

**Fig. 6. Two-hybrid analysis.** Yeast cells were co-transformed with a pGBT9-based bait vector and a pGAD10-based prey vector fused to full-length DVLP or its deletion mutants as shown in Fig. 1. A, the transformed cells were streaked on His-containing (+His) or His-deficient (−His) plates. The −His plate contained 25 mm 3-aminotriazole. B, the transformed cells were subjected to liquid β-Gal assay as described under “Experimental Procedures.”
DVLP(490–736) by the two-hybrid system. As shown in Fig. 6B, DVLP(1–424), which contains the largest part of DVH1 domain, was able to interact with DVLP(490–736). By contrast, DVLP(1–343), which lacks the DVH1 domain, was unable to interact with DVLP(490–736). These data indicate that the DVH1 domain is required for the interaction with the C-terminal DVH2-containing region. However, in addition to the DVH1 domain, the GTPase domain appears to stimulate the interaction, because DVLP(295–527), which lacks the GTP-binding domain, failed to show a significant interaction with DVLP(490–736).

We then examined which region of DVLP(490–736) is responsible for the interaction with DVLP(1–489). DVLP(490–634) was unable to interact with DVLP(1–489), indicating that the DVH2 domain is required for the interaction with the N-terminal region. We then truncated successively the DVLP(490–736) construct from its N terminus (Fig. 1). As shown in Fig. 6B, although DVLP(542–736) was capable of interacting with DVLP(1–489), DVLP(580–736) interacted no longer with the N-terminal region. These data indicate that although it is not conserved in the dynamin and Vps1 subfamilies, the region upstream of the DVH2 domain is also required for the interaction with the N-terminal region.

**DISCUSSION**

DVLP shares some primary structural features with dynamin. In this study, we have shown that, as is the case for dynamin, DVLP may be tetrameric under physiological salt conditions and aggregates into sedimentable complexes under low salt conditions. The similarities not only in the structure but also in the complex formation make it tempting to speculate that DVLP plays a role in the formation of carrier vesicles in a manner similar to that of dynamin, although it is currently unclear which transport step(s) DVLP is involved in. Under the appropriate conditions, DVLP tetramers in the cytosol may assemble into rings and/or spirals at the necks of budding coated pits prior to vesicle fission. In this context, a recent report of Carr and Hinshaw (20) is noteworthy. They have shown that even under physiological salt conditions, dynamin polymerizes into spirals in the presence of GDP and γ-phosphate analogues (aluminum fluoride and beryllium fluoride) or in the presence of GTPγS. We performed similar experiments for DVLP but failed to detect its assembly into sedimentable complexes in the presence of the GTP analogues under physiological salt conditions (data not shown). The reason for the difference between the behavior of dynamin and the behavior of DVLP is unclear. One possibility is that the difference may be caused by the difference in the used experimental system; Carr and Hinshaw (20) used purified recombinant dynamin, whereas we used crude cytosolic extract containing DVLP. Indeed, using the cytosolic fraction of Clone 9/DVLP-HA cells, we could not detect sedimentable complexes of dynamin II in the presence of the GTP analogues under physiological salt conditions (data not shown). To address this issue, experiments using purified DVLP will be required. Attempts are under way to express and purify recombinant DVLP using a baculovirus system.

Analyses using the two-hybrid system have shown the intermolecular interaction of DVLP and delineated domains of DVLP responsible for the interaction; the N-terminal region containing the GTPase and DVH1 domains can interact with the C-terminal DVH2-containing region. Because these three domains are highly conserved in the dynamin and Vps1 subfamilies, a common mechanism may govern the interdomain interactions of dynamin and DVLP. Indeed, our data are in good agreement with recent data on intermolecular and interdomain interactions of dynamin (19, 20, 24): (i) a dynamin mutant lacking the C-terminal Pro-rich domain, which is not present in DVLP, can form itself into tetramers (19) and assemble into spirals (20); (ii) an N-terminal fragment of dynamin that is deduced to contain the GTPase and DVH1 domains can interact with the C-terminal DVH2-containing region; (iii) the C-terminal DVH2-containing proteolytic fragments can be cross-linked with each other (19) and co-assemble into spirals (19, 20); and (iv) the pleckstrin homology domain, which is not present in DVLP, is not required for the co-assembly nor cross-linked with the N-terminal or DVH2-containing fragment (19). In this context, it is also noteworthy that the DVH2-containing fragment appears to stimulate the
GTPase activity of the N-terminal fragment (19). The interaction between the region containing the GTPase domain and the DVH2 domain may regulate the assembly/disassembly of the dynamin family proteins.

On the basis of the present data, three models for the tetramer formation of DVLP are possible (Fig. 9). In the first model (model 1), an interaction between the N- and C-terminal regions occurs in the same DVLP polypeptide, and the four monomer units then assemble into a tetramer. In the second (model 2), the interaction occurs between two adjacent antiparallel polypeptides, and the two dimer units then assemble into a tetramer. In the third (model 3), the interactions sequentially occur between adjacent parallel polypeptides, and a ring-like structure composed of four polypeptides is thereby formed. We favor the last model, because we could not detect any homotypic interactions between domains of DVLP by the two-hybrid analysis or any dimeric intermediates by the gel filtration and cross-linking analyses. In contrast, Muhlberg et al. (19) favored model 2 for the tetramer formation of dynamin on the basis of their analyses using its proteolytic fragments. A difference between our data on DVLP using the two-hybrid system and the data on dynamin using proteolytic fragments is that in the latter, homotypic interactions between the N-terminal fragments and between the DVH2-containing fragments were also detected, although to a much lesser extent. Despite the difference, the data of Muhlberg et al. (19) appear to be also compatible with model 3, which they did not propose. Otherwise, the mechanisms underlying the tetramer formation of DVLP and dynamin may be slightly different from each other, because, unlike dynamin (18, 19), no dimeric intermediates of DVLP were observed in the cross-linking experiments (Fig. 4).

REFERENCES
1. Vallee, R. B., and Okamoto, P. M. (1995) Trends Cell Biol. 5, 43–47
2. Liu, J.-P., and Robinson, P. J. (1995) Endocr. Rev. 16, 590–607
3. Damke, H. (1996) FEBS Lett. 389, 48–51
4. Urrutia, R., Henley, J. R., Cook, T., and McNiven, M. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 377–384
5. Shin, H.-W., Shirotaka, C., Torii, S., Murakami, K., and Nakayama, K. (1997) J. Biochem. 122, 525–530
6. Kamiyoto, T., Nagai, H., Onogi, H., Muro, Y., Wakabayashi, T., and Hagiwara, M. (1998) J. Biol. Chem. 273, 1044–1051
7. Yoon, Y., Pitts, K. R., Dahan, S., and McNiven, M. A. (1998) J. Cell Biol. 140, 779–783
8. Imoto, M., Tachibana, I., and Urrutia, R. (1998) J. Cell Sci. 111, 1341–1349
9. van der Bliek, A. M., Reddelmeier, T. E., Damke, H., Tisdale, E. J., Meyerowitz, E. M., and Schmid, S. L. (1993) J. Cell Biol. 122, 553–563
10. Herskovits, J. S., Burgess, C. C., Obar, R. A., and Vallee, R. B. (1993) J. Cell Biol. 122, 565–578
11. Damke, H., Baba, T., Warnock, D. E., and Schmid, S. L. (1994) J. Cell Biol. 127, 915–934
12. Kosaka, T., and Ikeda, K. (1983) J. Cell Biol. 97, 499–507
13. Jones, S. M., Howell, K. E., Henley, J. R., Cao, H., and McNiven, M. A. (1998) Science 279, 573–577
14. Takei, K., McPherson, P. S., Schmid, S. L., and De Camilli, P. (1995) Nature 374, 186–190
15. Hinshaw, J. E., and Schmid, S. L. (1995) Nature 374, 190–192
16. Rothman, J. H., Raymond, C. K., Gilbert, T., O’Hara, P. J., and Stevens, T. H. (1990) Cell 61, 1063–1074
17. Gammie, A. E., Kuribara, L. J., Vallee, R. B., and Rose, M. D. (1995) J. Cell Biol. 130, 553–566
18. Tuma, P. L., and Collins, C. A. (1995) J. Biol. Chem. 270, 26707–26714
19. Muhlberg, A. B., Warnock, D. E., and Schmid, S. L. (1997) EMBO J. 16, 6676–6683
20. Carr, J. F., and Hinshaw, J. E. (1997) J. Biol. Chem. 272, 28030–28035
21. Warnock, D. E., Baba, T., and Schmid, S. L. (1997) Mol. Biol. Cell 8, 2553–2562
22. Torii, S., Banno, T., Watanabe, T., Ikehara, Y., Murakami, K., and Nakayama, K. (1995) J. Biol. Chem. 270, 11574–11580
23. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) Current Protocols in Molecular Biology, pp. 13.6.2–13.6.5, John Wiley & Sons, New York
24. Lin, H. C., Barylko, B., Achiriloaie, M., and Albanesi, J. P. (1997) J. Biol. Chem. 272, 25999–26004

FIG. 9. Models for tetramer formation of DVLP. GBD, GTP-binding domain.