Dynamin 2 Is Required for Phagocytosis in Macrophages

By Elizabeth S. Gold,* David M. Underhill,* Naomi S. Morrissette,* Jian Guo,* Mark A. McNiven,§ and Alan Aderem*

From the *Department of Immunology and the ‡Division of Cardiology, University of Washington, Seattle, Washington 98195; and the §Department of Biochemistry and Molecular Biology, The Center for Basic Research in Digestive Diseases, Mayo Foundation, Rochester, Minnesota 55905

Summary

Cells internalize soluble ligands through endocytosis and large particles through actin-based phagocytosis. The dynamin family of GTPases mediates the scission of endocytic vesicles from the plasma membrane. We report here that dynamin 2, a ubiquitously expressed dynamin isoform, has a role in phagocytosis in macrophages. Dynamin 2 is enriched on early phagosomes, and expression of a dominant-negative mutant of dynamin 2 significantly inhibits particle internalization at the stage of membrane extension around the particle. This arrest in phagocytosis resembles that seen with inhibitors of phosphoinositide 3-kinase (PI3K), and inhibition of PI3K prevents the recruitment of dynamin to the site of particle binding. Although expression of mutant dynamin in macrophages inhibited particle internalization, it had no effect on the production of inflammatory mediators elicited by particle binding.

Key words: phagocytosis • macrophages • dynamin • inflammation • phosphoinositide 3-kinase

Macrophages are “professional phagocytes” that play a critical role in innate and acquired immunity due to their unique ability to internalize and degrade pathogens and to couple this to the release of inflammatory mediators. Phagocytosis is initiated by the interaction of specific receptors on the surface of the phagocyte with ligands on the particle. Although all phagocytosis requires actin polymerization, phagocytosis mediated through different receptors uses distinct mechanisms and results in different biological outcomes (1). Thus, macrophage phagocytosis of pathogens initiates inflammation, whereas the phagocytosis of apoptotic cells does not initiate a proinflammatory response (2). The specific molecular regulators of particle internalization and the mechanisms of coupling to the production of inflammatory mediators are largely unknown.

While cells use phagocytosis to internalize large particles, soluble ligands are internalized through receptor-mediated endocytosis, a clathrin-based process. Dynamin 2 is a ubiquitously expressed GTPase that has a critical role in the scission of forming clathrin-coated endocytic vesicles from the plasma membrane (3). Flies with a temperature-sensitive mutation in shibire, the Drosophila homologue of dynamin, have impaired endocytosis at the synaptic junction that results in their rapid paralysis at the nonpermissive temperature (4, 5). The nerve terminals of these mutant flies are depleted of synaptic vesicles and have an accumulation of partially invaginated coated pits at the cell surface (6). This defect in endocytosis is also found in several other tissues in these flies (7–9).

In mammalian cells, dominant-negative mutant forms of dynamin that are unable to bind GTP inhibit receptor-mediated endocytosis (10–12). When permeabilized nerve termini are treated with the nonhydrolyzable GTP analogue, GTPγS, tubular membrane invaginations coated with helical arrays of dynamin are formed (13). Similarly, dynamin assembles into collar-like rings around the neck of the tubular liposomes, and hydrolysis of GTP by dynamin leads to an active scission of these tubules into discrete vesicles (14–16). The precise mechanism by which dynamin functions in vesicle scission is controversial; some evidence supports dynamin acting as a mechanical force generator (13–15), whereas other data suggest that it acts as a classical GTPase switch that activates a downstream effector (17).

Dynamin 2 is also involved in membrane traffic at the trans-Golgi network (TGN). A neutralizing antibody directed against dynamin 2 inhibits the formation of both clathrin- and non–clathrin-coated vesicles at the TGN in vitro (18). There is also strong evidence that the Saccharomyces cerevisiae dynamin homologue, Vps1p, modulates vesicular trafficking from the TGN (19).

Dynamin 2 is targeted to forming endosomes through its interaction with the Src homology (SH) 3 domain of amphiphysin (20–22). Thus, overexpression of the SH3 do-

Abbreviations used in this paper: eGFP, enhanced GFP; GFP, green fluorescent protein; LDL, low-density lipoprotein; PI3K, phosphoinositide 3-kinase; RP, resident peritoneal; SH, Src homology; TGN, trans-Golgi network; TRITC, tetramethyl rhodamine isothiocyanate.
main of amphiphysin blocks receptor-mediated endocytosis at nerve terminals and in Cos-7 cells (23, 24). We recently cloned amphiphysin from an expression library using an mAb generated against mouse macrophage phagosomes, and have shown that amphiphysin is enriched on phagosomes (our unpublished results). This suggested a possible role for dynamin in phagocytosis. We report here that dynamin 2 localizes to forming phagosomes, and that a mutant form of dynamin 2 inhibits phagocytosis at the stage of membrane extension around the particle, but does not impair particle-mediated stimulation of inflammatory mediators.

**Materials and Methods**

**DNA Expression Vectors.** Full-length dynamin 2 (aa isoform) with a single amino acid mutation that changed the lysine at position 44 to an alanine, dynK44A, was cloned into the pT1G2 vector. In this vector, expression of dynK44A is under the control of a tetracycline-repressible promoter. Removal of tetracycline from the media results in a bicistronic mRNA that concomitantly directs translation of the dominant-negative dynamin protein and green fluorescent protein (GFP). pT1G2 consists of pcDNA3.1/Zeo (Invitrogen) in which the CMV promoter was replaced by the tetracycline-regulated promoter from ptetSplice (Xhol-HindII fragment; Gibco BRL) followed by a multiple cloning site, the cap-independent translational enhancer region of pCITE (amplified using the 5' primer, GTGGATCCGTTATTTTCCACCATATT, and the 3' reverse primer, GGGAGCTCCCATATTATCATCGTGT; Novagen) and the coding region for enlarged GFP (eGFP) from pEGFP-N1 (EcorI-N-otf fragment; Clontech).

V5 epitope-tagged dynamin 2 and dynK44A were constructed by TA cloning into the pcDNA3.1/V5/His/SPO vector (Invitrogen).

pNeo/Tak was constructed to direct expression of the tetracycline transactivator under neomycin selection. The plasmid uses a tetracycline-regulated promoter to direct expression of the tetracycline transactivator (both from ptet-Tak; Gibco BRL). The neomycin resistance marker was from pcDNA3 (Invitrogen), and the remainder of the plasmid was derived from pBluescript SK (Stratagene).

**Immunofluorescence Characterization.** Mouse resident peritoneal (P) macrophages were isolated and cultured as described previously (1). Synchronized phagosomes were created by centrifuging RP macrophages were isolated and cultured as described previously (25). Neomycin resistance was lost from pcDNA3 (Invitrogen) and all manipulations for this study were performed in the absence of neomycin.

**Immunoblotting.** Macrophages, either RP or RAW 264.7 cells (American Type Culture Collection), were lysed on ice by lysis buffer (20 mM Tris, pH 7.4, 120 mM NaCl, 1% SDS, 10% glycerol, 2 mM EDTA, 1 mM diethiothreitol, and 1% Triton X-100 for 1 h). Samples were quantified by a bicinchoninic acid protein assay (Pierce Chemical Co.), and 20 µg of protein was run on a 10% SDS-PAGE acrylamide gel. Proteins were transferred to polyvinylidene fluoride (PVDF) membrane (Millipore) (Milipore) and blocked overnight at 4°C in 10% nonfat dried milk in PBS. Membranes were incubated for 1 h at room temperature with one of the anti-dynamin antibodies (either Dyn 2 [25] or the anti-dynamin 1 rabbit polyclonal antibody DG2, provided by Pietro De Camilli, Yale University School of Medicine, New Haven, CT), washed three times for 15 min each in TBS/Tween and incubated in a 1:10,000 dilution of peroxidase-conjugated secondary antibody (Cappel). After three 15-min washes in TBS/Tween, specific binding was detected using chemiluminescence (Amerham Pharmacia Biotech).

**Cell Surface Staining.** Cells to be stained for FcγRII and FcγRIII were resuspended into FACScan buffer (PBS, 2% FCS, 0.5% azide) while cells to be stained for Mac-1 were resuspended into 2.4G2 supernatant (American Type Culture Collection), then incubated for 15 min on ice. Primary antibody (biotinylated 2.4G2 for Fcγ staining or biotinylated anti-mouse CD11b antibody, both from Pharmingen) was added, and the cells were incubated on ice for 20 min. Cells were washed in FACScan buffer, resuspended in diluted streptavidin-PE (Caltag), and incubated on ice for 15 min. The cells were washed, resuspended in FACScan buffer with 1% paraformaldehyde, and analyzed on a FACScan™ (Beckton Dickinson).

Transfections. A tetracycline transactivator-expressing RAW cell line was generated by transfecting RAW 264.7 cells with pNeo/Tak, and stable cell lines were selected using 400 µg/ml G418 (Gibco BRL). After 10 d of selection, the cells were cloned by limiting dilution, and one cell line (designated RAW-TT10) that demonstrated good tetracycline-regulated expression from a subsequently transfected reporter plasmid was used for all experiments. In the experiments reported here, tetracycline was always absent from the media, resulting in strong activity of the tetracycline-regulated promoter.

RAW-TT10 cells were transiently transfected by electroporation. 10 µg of DNA was added to 5 × 10^6 RAW-TT10 cells in 250 µl of RPMI (JRH Biosciences) with 10% heat-inactivated FBS.
FCs (Hyclone). The cells were electroporated at 280 V, capacitance 960 μF, and immediately washed in 5 ml of RPMI with 10% FCS. The cells were plated and analyzed 18–24 h later by FACS® or confocal microscopy.

Phagocytosis Assay. Tetramethyl rhodamine isothiocyanate (TRITC)-zymosan was purchased from Molecular Probes TRITC-labeled SR BC “ghosts” were prepared by incubating SR BCs (ICN/Cappel) in hypotonic lysis buffer (1 mM MgCl₂, 100 μM EGTA in 0.02× PBS) with TRITC-BSA (Molecular Probes) on ice for 1 h. Isotonicity was restored to the cells with 5× PBS, and the ghosts were resealed at 37°C for 1 h. U. Nuncorporated TRITC-BSA was removed by washing in PBS, and the ghosts were opsonized as described above (anti-SR BC IgG and IgM were purchased from Intercell).

The specific particles were centrifuged onto the transiently transfected RAW-TT10 cells at 1,600 rpm and 4°C for 1 min. (Before exposure to C3b-opsonized particles, cells were treated with 200 nM PMA for 30 min.) The cells were incubated at 37°C for 10 min. The extracellular particles were removed, ghosts were lysed with a 20-s water wash, and the TRITC-zymosan was digested for 10 min with 100 U/ml lyticase (Sigma Chemical Co.). The cells were resuspended in PBS/EDTA, fixed with 1% formalin, and analyzed by FACS®.

The effect of dynamin 1 on phagocytosis was assessed with three dominant-negative mutants of dynamin 1 expressed in pcMV5: D1ΔPH (deletion of amino acids 541–618), D1 K535M, and D1 N272 (deletion of amino acids 1-272) (28). Each of these constructs was cotransfected with pSFV-eGFP (eGFP under the control of the spleen focus-forming virus LTR) into RAW-TT10 cells, and phagocytosis was assayed as described above.

FACS Assay. RAW-TT10 cells were transfected with dynK44A, pT1GZ2 18 h before the assay. The cells were incubated with the indicated particles at 37°C for 30 min, at which time 5 μM brefeldin A was added to the media. The zymosan particles were endotoxin free as assessed by the limulus amebocyte lysate assay (QCL-1000; BioWhittaker). The cells were incubated for an additional 2.5 h, then collected for staining. The cells were blocked (QCL-1000) and stained with anti–TNF-α antibody (PharMingen) conjugated to PE in permeabilization mix (DI®). The cells were resuspended in PBS/EDTA, fixed with 1% formalin, and analyzed by FACS®.

Results
Dynamin 2 is enriched on Early Phagosomes in Macrophages. Dynamin 2 was found to be expressed in murine R.P macrophages and the RAW-TT10 macrophage cell line, whereas dynamin 1 was not detected in macrophages (Fig. 1A). Interestingly, dynamin 2 was recruited to early phagosomes, as demonstrated by staining with two independent antibodies to dynamin as well as by the localization of the epitope-tagged protein (Fig. 1B). The phagocytosis of different particles is mediated by different receptors. For example, FcRs mediate the uptake of IgG-coated particles, complement receptors (CRs) mediate the uptake of C3b-opsonized particles, and the mannose receptor (among others) mediates the uptake of zymosan (yeast cell wall particles) (2). Phagocytosis stimulated by these receptors has common features, such as a reliance on the actin cytoskeleton, and distinct features, such as their different requirement for the cytoskeletal proteins vinculin and paxillin (1). Immunofluorescence microscopy of murine R.P macrophages using two independent antibodies demonstrated that dynamin 2 was enriched on early phagosomes containing IgG-opsonized RBCs, C3b-opsonized RBCs, and zymosan (Fig. 1B, i, iii, vii, and viii). In addition, an epitope-tagged version of the aa isoform of dynamin 2 transiently expressed in RAW-TT10 macrophages also localized to phagosomes, demonstrating that this macrophage-expressed isoform contains the domain responsible for targeting to phagosomes (Fig. 1B, iv and v). The kinetics of association of dynamin 2 with phagosomes precisely mirrored that of F-actin: both were recruited to the forming phagocytic cup and the early phagosome (Fig. 1B, v and vii), and both were concomitantly lost from the phagosome after particle internalization (data not shown).

Dynamin 2 is Critical for Macrophage Phagocytosis. To examine the role of dynamin 2 in phagocytosis, RAW-TT10 macrophages were transfected with a dominant-negative mutant form of the aa isoform of dynamin 2, dynK44A, which is unable to bind GTP (11). The mutant dynamin 2 gene was expressed in a bicistronic vector with GFP (pT1G2 vector), allowing transiently transfected cells to be identified by their green fluorescence. Phagocytosis of either TRITC-labeled zymosan, IgG-opsonized RBCs, or C3b-opsonized RBCs was assessed as a function of the level of expression of the GFP/dominant-negative protein by two-color FACS® (Fig. 2). In all cases, dynK44A inhibited phagocytosis in a dose-dependent manner (Fig. 2A); a typical FACS® profile is shown in Fig. 2B. DynK44A inhibited FcR-mediated phagocytosis by 85%, CR-mediated phagocytosis by 63%, and zymosan phagocytosis by 65% (Fig. 2C). As expected, dynK44A also inhibited receptor-medi-
Dynamin 2 is enriched on murine macrophage phagosomes (A) 20 μg of total protein extract from RP macrophages (PMφ), RAW-TT10 macrophages, or brain was separated on a 10% SDS-PAGE gel, transferred to polyvinylidene difluoride membrane, and probed with an anti-dynamin 2 (Dyn 2), or an anti-dynamin 1 (DG2) antibody as indicated. A protein of the expected 100-kD size was detected in macrophages by the anti-dynamin 2 antibody but was not detected by the anti-dynamin 1 antibody. (B) Synchronized phagosomes containing either zymosan particles (i–vi), complement-opsonized RBCs (vii), or IgG-opsonized RBCs (viii) were created in RP macrophages (i, ii, iii, vii, viii) or in transiently transfected RAW-TT10 cell expressing V5 epitope–tagged dynamin 2 (i, vii, and viii) or in transiently transfected RAW-TT10 cell expressing V5 epitope–tagged dynamin 2 (i–vi). 5 min after particle binding, cells were fixed and prepared for immunofluorescence. Dynamin 2 was detected with the anti-dynamin 2 antibody Dyn 2 (i, vii, and viii) or the pan anti-dynamin antibody MC63 (iii); V5 epitope–tagged dynamin was detected with anti-V5 antibody (iv and v); actin was stained with rhodamine-phalloidin (vi); and zymosan was visualized directly (ii). Dynamin enrichment on phagocytic cups and phagosomes (v) colocalized with F-actin (vi).

Figure 2. DynK44A inhibits macrophage phagocytosis. Transiently transfected RAW-TT10 macrophages internalized the indicated particles for 10 min. Uninternalized particles were removed, and the cells were analyzed by flow cytometry. (A) DynK44A inhibited phagocytosis in a dose-dependent manner. DynK44A expression, as determined by GFP fluorescence, is plotted on a logarithmic scale on the x-axis. Phagocytosis, expressed as the percentage of cells internalizing particles relative to the percentage of nonexpressing control cells internalizing particles is plotted on the y-axis. The SEM from four independent experiments is shown, but the error bars do not extend beyond the symbols. (B) A typical experiment is shown. RAW-TT10 cells transfected with dynK44A-pTIGZ2 were incubated with TRITC-zymosan and analyzed. The level of transgene expression is expressed on the x-axis (GFP fluorescence), and the phagocytosis of labeled particles is shown on the y-axis (TRITC fluorescence). (C) RAW-TT10 cells were transiently transfected with either pTIGZ2 vector alone (control) or dynK44A-pTIGZ2, and phagocytosis of TRITC–loaded IgG-opsonized SRBCs, TRITC–loaded complement-opsonized SRBCs, or TRITC–zymosan was assessed. Phagocytosis is expressed as the percentage of transfected cells internalizing particles relative to the percentage of untransfected cells internalizing particles. Control and dynK44A cells expressing the same level of GFP were compared. The data shown represent a minimum of three independent experiments, and error bars reflect SEM.
Dynamin 2 blocks receptor-mediated endocytosis in macrophages. RAW-TT10 cells, transfected with the TIGZ control vector (thin solid line) or the dynK44A vector (thick solid line), were incubated with 20 µg/ml of Dil-labeled acetylated LDL (Dil AcdLDL) for 30 min at 37°C. As a control, untransfected cells were incubated with the same concentration of Dil AcdLDL at 4°C (dashed line). Cells were collected and analyzed by flow cytometry. The amount of intracellular Dil AcdLDL is indicated on the x-axis in log fluorescence units. The histograms were generated by gating on the highly expressing transfected cells (see Fig. 2 B for sample gates). The gates used in this experiment were the same as those used to assay phagocytosis.

Dynamin 2 is required for membrane extension around the forming phagosome. Our initial hypothesis was that dynamin would serve a similar role in phagocytosis as it serves in endocytosis and therefore would be required only for scission of the nascent phagosome from the plasma membrane. However, examination of dynK44A-expressing RAW-TT10 cells attempting to internalize particles revealed that dynamin was exerting a role earlier in the process. Cells expressing dynK44A were able to bind particles, and phalloidin staining demonstrated that this was accompanied by localized actin polymerization; however, actin extended only partially around the particles (Fig. 4). To determine the stage at which phagocytosis was arrested, dynK44A-expressing RAW-TT10 cells were studied by scanning electron microscopy. 10 min after contacting IgG-coated SRBCs, control cells (expressing pTIGZ2 alone) were identified at many different stages of particle internalization (Fig. 5 A), while very few of the dynK44A-expressing cells extended membrane more than halfway around the SRBCs (Fig. 5 B). This indicated that mutant dynamin arrested particle internalization at an intermediate stage. Indeed, after unbound SRBCs were washed away and phagocytosis was allowed to proceed for an additional 50 min at 37°C, the pTIGZ2 control cells had internalized >90% of the particles (Fig. 5 C, and Table I). In contrast, <30% of the particles associated with the dynK44A-expressing cells were internalized (Fig. 5 D, and Table I).

PI3K is a key regulator of macrophage phagocytosis (29, 30). As a control, untransfected cells were incubated with the same concentration of Dil AcdLDL at 4°C (dashed line). Cells were collected and analyzed by flow cytometry. The amount of intracellular Dil AcdLDL is indicated on the x-axis in log fluorescence units. The histograms were generated by gating on the highly expressing transfected cells (see Fig. 2 B for sample gates). The gates used in this experiment were the same as those used to assay phagocytosis.

In addition, mutant dynamin had no effect on particle-induced TNF-α production (see Fig. 7, below), demonstrating that one arm (internalization) of a bifurcating signaling pathway was selectively inhibited.

**Table I.** Bound, Incompletely Internalized Particles

|          | Bound particles |
|----------|-----------------|
|          | 10 min          | 1 h            |
| Control  | 450 ± 37        | 35 ± 10        |
| DynK44A  | 480 ± 53        | 300 ± 28       |

Data represent number of bound, incompletely internalized IgG SRBCs per 100 macrophages examined by scanning electron microscopy. Error is SEM from three samples. Examination of parallel samples by fluorescence confirmed that after 1 h, >90% of the particles on the control cells were internalized, whereas <30% of the particles associated with the dynK44A-expressing cells were internalized.
Inhibition of PI3K causes incomplete phagosome closure (29, 30), a very similar phenotype to that observed in cells expressing mutant dynamin. Inhibition of PI3K with wortmannin prevented the recruitment of dynamin 2 to the site of particle binding and actin polymerization (Fig. 6, A and B). Thus, it is possible that PI3K might act upstream of dynamin in phagocytosis.

Dynamin 2 Selectively Uncouples Particle Internalization from Particle-induced Cytokine Production. Macrophage phagocytosis of IgG-coated particles and zymosan results in several signaling events, including the production of inflammatory mediators such as TNF-α (2). Cells expressing dynK44A generated normal amounts of TNF-α upon interaction with particles (Fig. 7); thus, mutant dynamin uncoupled particle internalization from particle-dependent cytokine production.

Discussion

In this study, we have demonstrated that dynamin 2 is essential to the formation of macrophage phagosomes, and that it functions at the stage of membrane extension around the particle. This role for dynamin is conserved in all of the...
The phagocytic defect induced by dynK44A resembles that seen when PI3K is inhibited in macrophages (29, 30). This is of interest, since dynamin interacts with the p85 regulatory subunit of PI3K and this interaction stimulates dynamin’s GTPase activity (31). We report here that inhibition of PI3K prevents the recruitment of dynamin 2 to the site of particle binding, suggesting that the activation of PI3K is upstream of dynamin in mediating phagocytosis. PI3K supports phagocytosis in macrophages, in part, by facilitating the insertion of membrane into forming phagosomes (30). The scanning electron micrographs shown here suggest that membrane extension may also be the stage of arrest in the cells expressing dynK44A. Membrane extension is known to require the fusion of vesicles with the plasma membrane (30, 32–35); thus, it is tempting to speculate that dynamin’s role in phagocytosis is related to its capacity to recruit membrane to nascent phagosomes. In support of this, the yeast homologue of dynamin, Vps1p, is required for bidirectional trafficking between endosomes and the vacuole (19).

Although our data suggest a role for dynamin 2 in extending membrane around the nascent phagosome, it does not rule out other mechanisms for dynamin’s effect on phagocytosis. For example, dynamin might have a direct effect on actin during phagosome formation, since it has been demonstrated to interact with profilin, an actin-binding protein (36). It remains possible that dynamin is also involved in the scission of the neck behind the phagosome, similar to its known role in endocytosis. However, we have not observed any enrichment of dynamin at the scission site of the phagosome.

The phagocytosis of pathogens by macrophages is tightly coupled to the elaboration of inflammatory cytokines that, in turn, orchestrate an appropriate immune response. It has long been known that particle binding by macrophages induces actin-mediated internalization and inflammatory mediator production through a bifurcating signaling cascade (37, 38). Dynamin clearly regulates the particle internalization limb of this pathway while it has no role in the production of inflammatory cytokines.

We thank Dr. Pietro De Camilli for providing the antibody to dynamin 1, Dr. Joseph Albanesi (University of Texas Southwestern Medical Center, Dallas, TX) for providing expression vectors containing the dominant-negative dynamin 1 mutations, Jessica Hamerman and Adrian Ozinsky for critical review of the manuscript, Stephanie Lara for help with scanning electron microscopy, and Kathy Allen for operating the FACStar™.

This work was supported by grant AI-R37-25032 and AI-R01-32972 from the National Institutes of Health. E.S. Gold was supported by grants from the American Heart Association and the Cardiofellow Foundation, and D.M. Underhill is an Irvington Institute postdoctoral fellow.

Address correspondence to Alan Aderem, Department of Immunology, Box 357650, 1959 N.E. Pacific Ave., Seattle, WA 98195. Phone: 206-616-5045; Fax: 206-616-7237; E-mail: aaderem@u.washington.edu

Submitted: 24 August 1999 Revised: 6 October 1999 Accepted: 7 October 1999

References
1. Allen, L.-A., and A. Aderem. 1996. Molecular definition of distinct cytoskeletal structures involved in complement- and Fc receptor-mediated phagocytosis in macrophages. J. Exp. Med. 184:627–637.
2. Aderem, A., and D.M. Underhill. 1999. Mechanisms of phagocytosis in macrophages. Annu. Rev. Immunol. 17:593–623.
VanderBliek, A.M., and E.M. Meyerowitz. 1991. Dynamin-like protein encoded by the Drosophila shibire gene associated with vesicular traffic. Nature. 351:411–414.

Kosaka, T., and K. Ikeda. 1983. Possible temperature-dependent blockage of synaptic vesicle recycling induced by a single gene mutation in Drosophila. J. Neurobiol. 14:207–225.

Kesseli, I., B.D. Holst, and T.F. Roth. 1989. Membrane intermediates in endocytosis are labile, as shown in a temperature-sensitive mutant. Proc. Natl. Acad. Sci. USA. 86:4968–4972.

Koenig, J.H., and K. Ikeda. 1990. Transformational process of the endosomal compartment in nephrocytes of Drosophila melanogaster. Cell Tissue Res. 262:233–244.

Kosaka, T., and K. Ikeda. 1983. Reversible blockage of membrane retrieval and endocytosis in the garland cell of the temperature-sensitive mutant of Drosophila melanogaster, shibire. J. Cell Biol. 97:499–507.

Danke, H., T. Baba, D.E. Warrock, and S.L. Schmid. 1994. Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. J. Cell Biol. 127:915–934.

VanderBliek, A.M., T.E. Redelmeier, H. Danke, E.J. Tisdale, E.M. Moyerowitz, and S.L. Schmid. 1998. Dynamin's GAP domain stimulates receptor-mediated endocytosis. Proc. Natl. Acad. Sci. USA. 95:11691–11696.

Achiriloaeie, M., B. Barylko, and J.P. Albanesi. 1999. Essential role of the dynamin pleckstrin homology domain in receptor-mediated endocytosis. Mol. Biol. Cell. 19:1410–1415.

Araki, N., M.T. Johnson, and J.A. Swanson. 1996. A role for phosphoinositide 3-kinase in the completion of macropinosis and phagocytosis by macrophages. J. Cell Biol. 135:1249–1260.

Cox, D., C.-C. Tseng, G. Bjekic, and S. Greenberg. 1999. A requirement for phosphatidylinositol 3-kinase in pseudopod extension. J. Cell Biol. 141:85–99.

Henley, J.R., and M.A. McNiven. 1996. Association of a dynamin-like protein with the Golgi apparatus in mammalian cells. J. Cell Biol. 133:761–775.

Aderem, A.A., W.A. Scott, and Z.A. Cohn. 1984. A selective defect in arachidonic acid release from macrophage membranes in high potassium media. J. Cell Biol. 99:1235–1241.

Hackam, D.J., O.D. Rotstein, M.K. Bennett, A. Klip, S. Grinstein, and M.F. Manolios. 1996. Characterization and subcellular localization of target membrane soluble NSF attachment protein receptors (t-SNAREs) in macrophages. J. Immunol. 156:4377–4383.

Hackam, D.J., O.D. Rotstein, C. Sjolin, A.D. Schreiber, W.S. Trible, and S. Grinstein. 1998. v-SNARE-dependent secretion is required for phagocytosis. Proc. Natl. Acad. Sci. USA. 95:11691–11696.

Tapper, H., and S. Grinstein. 1997. Fc receptor-triggered insertion of secretory granaules into the plasma membrane of human neutrophils: selective retrieval during phagocytosis. J. Immunol. 159:409–418.

Aderem, A.A., W.A. Scott, and Z.A. Cohn. 1986. Evidence for sequential signals in the induction of the arachidonic acid cascade in macrophages. J. Exp. Med. 163:139–154.

Aderem, A.A., W.A. Scott, and Z.A. Cohn. 1984. A selective defect in arachidonic acid release from macrophage membranes in high potassium media. J. Cell Biol. 99:1235–1241.