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Syntaxins 13 and 7 Function at Distinct Steps During Phagocytosis

Richard F. Collins,* Alan D. Schreiber,† Sergio Grinstein,* and William S. Trimble**

The phagosome is a dynamic organelle that undergoes progressive changes to acquire the machinery required to kill and degrade internalized foreign particles. This maturation process involves sequential interaction of newly formed phagosomes with several components of the endocytic pathway. The proteins that mediate the ordered fusion of endosomes and lysosomes with the phagosome are not known. In this study, we investigated the possible role of syntaxins present in the endo/lysosomal pathway in directing phagosomal maturation. We show that in phagocytic cells syntaxin 13 is localized to the recycling endosome compartment, while syntaxin 7 is found in late endosomes/lysosomes. Both proteins are recruited to the phagosome, but syntaxin 13 is acquired earlier and rapidly recycles off the phagosome, while syntaxin 7 is recruited later and continues to accumulate throughout the maturation process. Overexpression of truncated (cytosolic) forms of syntaxin 13 or 7 had no effect on phagocytosis, but exerted an inhibitory effect on phagosomal maturation. These results indicate that syntaxins 13 and 7 are both required for interaction of endosomes and/or lysosomes with the phagosome, but play distinct roles in the maturation process. The Journal of Immunology, 2002, 169: 3250–3256.

Phagocytosis is a central component of the innate immune response, whereby specialized cell types recognize and engulf foreign extracellular material. Neutrophils and macrophages have the unique ability to ingest microorganisms, particularly after they are coated by opsonins, soluble host proteins such as complement and Ig, that are recognized by receptors on the surface of the phagocytes (1, 2). In the case of Ig-coated particles, receptors of the Fc family (FcR) bind to the opsonizing Ig, triggering a cascade of intracellular signaling events that leads to the ingestion of the particles (2–4). The phagosome then undergoes a maturation process, involving sequential interactions with other intracellular compartments, that culminates with the formation of a phagolysosome, in which the ingested particle is destroyed (1, 5, 6).

The formation of the phagosome requires the targeted delivery of vesicle-associated membrane protein (VAMP)3-containing vesicles from the recycling endosome compartment, contributing to the elongation of membranous pseudopods (7). VAMP-3 is a member of the family of soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins that are thought to be required for the fusion of vesicles with their target membranes. In the nervous system, members of the VAMP family present on vesicles form coiled-coil complexes with SNARE proteins from the syntaxin and SNAP-25 families located on the target membrane. The formation of these complexes is thought to provide the driving force for membrane fusion (8). Multiple members of the VAMP and syntaxin families have been identified, and each appears to localize to specific membrane compartments along the secretory and endocytic pathways. This has been taken as evidence that unique sets of SNAREs may mediate fusion between membranes from specific compartments.

Once internalized, the phagosomal membrane undergoes a progressive maturation, first resembling early endosomes and subsequently acquiring markers found in the late endosome and lysosome compartments (9). Maturation can be monitored by the phagosomal accumulation of endocytic proteins such as rab5 or the transferrin receptor at early time points, followed by the acquisition of late endosomal and lysosomal proteins such as rab7, the mannose 6-phosphate receptor, and lysosome-associated membrane protein (LAMP)-1. Interestingly, many of the early markers are lost as the late markers are being acquired, suggesting that this maturation process involves multiple ordered fusion/fission events with different components of the endocytic pathway (10).

In vitro studies have shown that phagosome-endosome and phagosome-lysosome fusions are dependent on the ATPase N-ethylmaleimide-sensitive factor (11, 12), indicating a requirement for SNARE proteins throughout this process. Moreover, it has been observed that the efficiency of defined fusion events with the isolated phagosome changes over time, suggesting that temporal changes in its membrane composition influence its capacity for fusion. Early phagosomes isolated within 20 min of the onset of phagocytosis fuse readily with endosomes, but poorly with lysosomes, while phagosomes that have matured for 2 h in vivo fuse most efficiently with lysosomes, but not at all with endosomes (13, 14).

Because SNARE proteins are required for phagosome-endosome and phagosome-lysosome fusion in vitro, and SNARE proteins such as syntaxin are localized to specific cellular compartments, it is reasonable to predict that specific SNARE proteins are required at different steps in phagosomal maturation in vivo. Moreover, if progressive changes in SNARE composition occur in vivo, this could explain differences in the propensity of phagosomes

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1 Abbreviations used in this paper: VAMP, vesicle-associated membrane protein; GFP, green fluorescent protein; EGFP, enhanced GFP; LAMP, lysosome-associated membrane protein; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; TM, transmembrane domain.
isolated at different stages of maturity to fuse with organelles in vitro. Therefore, we set out to investigate the role in phagosome maturation of specific isoforms of the syntaxin family implicated in the endocytic pathway. As a starting point, we examined syn-
taxins 13 and 7, the two best-characterized endosomal syntaxins. We show in this study that syntaxins 13 and 7 reside in different compartments in phagocytic cells and that they accumulate in the phagosome at different rates. Moreover, we show that inhibition of their function by overexpression of dominant-negative forms ar-
est the maturation process before the acquisition of lysosomal markers.

Materials and Methods

Reagents and Abs

SRBC and rabbit anti-SRBC IgG were purchased from ICN Pharmaceutical-
cs (Costa Mesa, CA). Polystyrene beads, 3.3 μm in diameter, were pur-
chased from Bangs Laboratories (Carmel, IN). Human IgG was purchased
from Sigma-Aldrich (St. Louis, MO). α-MEM and PBS were purchased from
Cellgro (Herndon, VA). FBS and G418 were from Wisen (St. Bruns-
que, Canada). HEPES-buffered RPMI (H-RPMI) was purchased from
Sigma-Aldrich. FuGene-6 transfection reagent was obtained from Hoff-
mann-LaRothe (Nutley, NJ). PEI25K (polyethylenimine, 25 kDa) was also
used as a transfection agent and came from Sigma-Aldrich. DAKO mount-
ing medium was from DAKO (Carpenteria, CA). Tetramethylrhodamine-
conjugated transferrin was purchased from Molecular Probes (Eugene,
OR). Restriction enzymes NheI, SacI, EcoRI, and Xhol were purchased from
Life Technologies (Burlington, Ontario, Canada). PFF DNA poly-
merase was obtained from Stratagene (La Jolla, CA).

Mouse anti-human transferrin receptor receptor was purchased from Zymed Lab-
oratories (San Francisco, CA). Rabbit Ab specific to mannansidase II was a
kind gift from K. Moreman (University of Georgia, Athens, GA) and M.
Farquhar (University of California, San Diego, CA). mAb to Golgi matrix
marker GM130 was purchased from Transduction Laboratories (Lexing-
ton, KY). Rabbit antimannose 6-phosphate receptor was a gift from S.
Hoening (Georg-August-Universität, Göttingen, Germany). Mouse anti-
lamp-1 and rat anti-lAMP-1 were obtained from the Developmental
Studies Hybridoma Bank (University of Iowa, Iowa City, IA). Anti-MYC
Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).
Donkey anti-mouse Cy3, anti-rabbit Cy3, anti-rat Cy3, and anti-human
Cy3 secondary Abs were purchased from Jackson Immunoresearch Lab-
oratories (West Grove, PA). Goat anti-mouse Alexa 488 and anti-rabbit
Alexa 488 secondary Abs were obtained from Molecular Probes.

cDNAs constructs

Syntaxin 7 and 13 cDNAs were a kind gift of R. Scheller (Stanford
University, Stanford, CA). N-terminal MYC-tagged syntaxin 7 was generated
in a two-step process. First, oligonucleotides encoding the MYC-C epitope (CTA
GAGCCACATGGGAGAAGC-TGATCCAGGGAAG-GAATTC
and CTACGCACTCCTCCTCTGGATCTAGGCTTT-GCGGACCTG
and GCGGAATTCGGTGGTTCAATCCCCATATGATGAGAC), digested
and ligated into pCDNA3.1 (Invitrogen, San Diego, CA), previously digested with
NheI, generating the construct MYC-N. Syntaxin 7 cDNA was PCR amplified
using PFF DNA polymerase and oligomers (GCCGAGCTCAAGATCGTC
and GCGGAAATTCGGTGGTTCAATCCCCATATGATGAGAC), digested
and ligation into the vector pEGFP-N1 (Clontech Laboratories, Palo Alto, CA), to create syntaxin 7-EGFP. The complete
syntaxin 7 gene was then excised with Xhol and EcoRI. This fragment was
ingenerated with an N-terminal MYC-tagged syntaxin 7 construct, subsequently referred to as 7 MYC. The fidelity of
MYC-N and syntaxin 7 cDNA was confirmed by DNA sequence analysis.

Syntaxin 13 cDNA was PCR amplified using PFF DNA polymerase and oligomers (GCCGAGCTCAAGATCGTC
and GCGGAAATTCGGTGGTTCAATCCCCATATGATGAGAC), digested
and ligation into the vector pEGFP-N1. PFCs were transfected with 2 μg DNA for 48 h,
and then incubated in 50 μg/ml rhodamine-transferin for 30 min at 37°C.

Cytosolic syntaxin 7 and 13 expression

Cos2A cells were plated to 50% confluence in α-MEM with 10% FCS
on glass coverslips. Cells were cotransfected with 0.1 μg EGFP mixed with
one of the following: 2 μg synactin 7 DNA (7Δ TM); 2 μg synactin 13 DNA
(13α TM); or 2 μg control DNA (pCDNA3.1). Cells were subse-
quently cocultured for another 48 h, as described above. For the transfection
experiments, cells were starved in α-MEM without FBS for 1 h, washed in PBS,
and then incubated in 50 μg/ml rhodamine-transferin for 30 min at 37°C.

Cytosolic syntaxin 7 (7Δ TM) was constructed from the syntaxin 7 EGFP construct using the QuikChange Site-
directed Mutagenesis kit from Stratagene Cloning Systems, to create a
translation stop codon immediately N terminal to the transmembrane domain.
Oligonucleotides ATCGACGCGGAAATCTAGATTTAACAACCTGG-TGAT and ATG
CACAGGTTTTACATGAGTTGGGGCTAG, were used and
mutants were screened for the introduction of a new Xhol site.

Cell culture and transfections

RAW 264.7 cells were obtained from the American Type Culture Collec-
tion (Manassas, VA). The COS-2A stable cell line, a COS-1 cell line ex-
pressing FcγRIIA, was described previously (4). Cells were selected in 1
μg/ml G418. For assays, RAW or COS-2A cells were grown to 50% con-
fluence on 25-mm glass coverslips in α-MEM medium supplemented with
10% FCS, at 37°C in 5% CO2. Transfection of cells with FuGene-6 was
according to manufacturer’s instructions. For immunofluorescence
studies and phagocytosis assays, 1 μg DNA and 3 μl FuGene-6 were used per
coverslip, 24 h before analysis. For dominant-negative studies using cyto-
solic syntaxin 7 or 13, cells were transfected with 2 μg DNA for 48 h,
before assessing. Some transfections were also performed using the reagent
PEI25K, as previously described (15), using 1 μg DNA per coverslip, with
similar results.

Indirect immunofluorescence and confocal microscopy

RAW cells were transfected with 7 MYC or 13GFP DNA and after 24 h
fixed in 4% paraformaldehyde/PBS at room temperature for 1 h, or
overnight at 4°C. Cells were permeabilized with 0.1% Triton X-100 in PBS
containing 100 mM glycine and blocked in 5% serum/PBS for 1 h. Primary
Abs were added at room temperature for 1 h in 1% serum at the following
dilutions: mouse anti-human transferrin receptor receptor (1:100), rabbit antimann-
osidase II (1:1000), monoclonal to Golgi matrix marker GM130 (1:500),
rabbit antimannose 6-phosphate receptor (1:800), rat anti-LAMP-1 (1:4),
rabbit anti-MYC (1:200), and rabbit anti-MYC (1:100). After incubation
with primary Abs, coverslips were washed in PBS and incubated with
secondary Abs in 1% serum for 30–60 min, as follows: donkey anti-rat
Cy3; anti-mouse Cy3 or anti-rabbit Cy3 (1:1000); goat anti-mouse Alexa
488 or anti-rabbit Alexa 488 (1:2000). Coverslips were washed in PBS, and
mounted using DAKO mounting medium. Imaging was performed on an
LSM510 Zeiss (Oberkochen, Germany) confocal microscope.

Phagocytosis of SRBC

SRBCs were opsonized with rabbit anti-SRBC IgG, at 37°C, for 1 h, fol-
lowed by washing in PBS. Transfected cells on coverslips were cooled to
4°C in H-RPMI and allowed to bind opsonized RBCs for 10 min, followed
by a PBS wash to remove unbound RBCs, then transferred to α-MEM
medium supplemented with 10% FCS and prewarmed to 37°C. Phagocy-
tosis was allowed to occur for various periods, as indicated. Phagocytosis
was stopped by transferring coverslips to H-RPMI precooled to 4°C, for 10
min. Coverslips were transferred to H-RPMI precooled to 4°C and con-
taining 1:1000 donkey anti-rabbit Cy5, to stain external RBCs, and allowed
to incubate for 15 min at 4°C. Coverslips were washed in ice-cold PBS
and fixed in 4% paraformaldehyde overnight. For cells transfected with 7
MYC, immunofluorescence was performed as described above, using
mouse anti-human transferrin receptor receptor (1:1000), followed by
green Alexa 488 (1:2000). For cells transfected with 13GFP, coverslips were washed in PBS
and mounted on slides.

Cytosolic syntaxin 7 or 13 expression

Cos2A cells were plated to 50% confluence in α-MEM with 10% FCS
on glass coverslips. Cells were cotransfected with 0.1 μg EGFP mixed with
one of the following: 2 μg synactin 7 DNA (7Δ TM); 2 μg synactin 13 DNA
(13α TM); or 2 μg control DNA (pCDNA3.1). Cells were subse-
quently cocultured for another 48 h, as described above. For the transfection
experiments, cells were starved in α-MEM without FBS for 1 h, washed in PBS,
and then incubated in 50 μg/ml rhodamine-transferin for 30 min at 37°C.

Cytosolic syntaxin 7 with the transmembrane domain (TM) (7Δ TM) was
made from the syntaxin 7 EGFP construct using the QuikChange Site-
directed Mutagenesis kit from Stratagene Cloning Systems, to create a
translation stop codon immediately N terminal to the transmembrane domain.
Oligonucleotides ATCGACGCGGAAATCTAGATTTAACAACCTGG-TGAT and ATG
CACAGGTTTTACATGAGTTGGGGCTAG, were used and
mutants were screened for the introduction of a new Xhol site.

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in H-RPMI at 4°C for 10 min. External beads were stained with donkey anti-human Cy5 IgG (1:1000) in H-RPMI at 4°C, 30 min. Cells were then washed in ice-cold PBS, fixed, permeabilized, and stained for LAMP-1.

**Quantitative image analysis**

After confocal microscopy images were obtained, densitometric measurements of LAMP-1 around the phagosome were made and the data were analyzed using Microcal Origin 6.0.

**Results**

Syntaxin 13 colocalizes with the transferrin receptor in the recycling endosome

Syntaxin 13 has been localized to tubular early and recycling endosomes in fibroblasts (16). To determine the location of syntaxin 13 in professional phagocytic cells, we set out to examine RAW cells. However, commercially available Abs to syntaxin 13 failed to detect the protein in these cells (data not shown). Therefore, we created an in-frame fusion between syntaxin 13 and EGFP in a mammalian expression vector in which the green fluorescent protein (GFP) was attached to the carboxyl terminus of syntaxin 13 (called 13GFP hereafter). This would locate EGFP in the luminal side of the endocytic membranes. To define the subcellular localization of syntaxin 13, RAW cells transfected with 13GFP were fixed after 24 h and then stained with Abs to markers of defined subcellular compartments. As shown in Fig. 1, there was significant overlap between the pattern of transferrin receptors (Fig. 1A) and that of 13GFP (Fig. 1B). In contrast, there was little, if any, overlap between 13GFP and the Golgi marker GM130 (Fig. 1, cf
C and D), the mannose 6-phosphate receptor (Fig. 1, cf E and F), or LAMP-1 (Fig. 1, cf G and H). Thus, as in fibroblasts, syntaxin 13 in phagocytic cells localizes with transferrin receptor-positive endosomes.

**Syntaxin 7 colocalizes with the mannose 6-phosphate receptor in the late endosome**

In a parallel set of experiments, we examined the localization of syntaxin 7 in RAW cells. Again, due to the lack of suitable Abs, it was necessary to tag the syntaxin 7. Preliminary experiments with a GFP tag similar to that used for syntaxin 13 failed, as a large percentage of the protein appeared to lack the GFP tag (not shown). Given the reported location of syntaxin 7 in the lysosome, we inferred that proteolysis of the luminal GFP may have occurred in this compartment. We therefore created a syntaxin 7 protein with an amino-terminal myc tag (called 7 MYC hereafter) that would be localized to the cytoplasm. This approach had previously been used successfully to localize syntaxin 7 in Madin-Darby canine kidney cells (17).

Transfected RAW cells were dual stained with Abs specific to the myc epitope and to specific cellular organelles. As shown in Fig. 2, 7 MYC differed from syntaxin 13 in that its distribution was distinct from that of the transferrin receptor (Fig. 2, A and B). Instead, 7 MYC overlapped significantly with both the mannose 6-phosphate receptor (Fig. 2, cf E and F) and LAMP-1 (Fig. 2, cf G and H). There was little, if any, overlap with the Golgi marker mannosidase II (Fig. 2, C and D). Hence, consistent with its localization in other cell types (18), syntaxin 7 appears to localize with the late endosome and lysosome compartments.

**Distinct time courses of syntaxin 13 and 7 accumulation in the phagosome**

To determine whether syntaxins 13 and 7 participate in phagosome formation or maturation, we next examined their location during the ingestion of opsonized particles. RAW cells were transfected with 13GFP or 7 MYC for 24 h, then exposed to SRBC that had been coated with rabbit anti-SRBC Abs. As can be seen in Fig. 3A, 13GFP is rapidly recruited to the nascent phagosomes within 2.5 min (arrows). In some cases, the accumulation of 13GFP appears to occur before the closure of the phagocytic cup. The presence of 13GFP begins to decline by 5 min (Fig. 3, B and F), and it is no longer seen around phagosomes after 10 min (Fig. 3, C and F), indicating that syntaxin 13 is only transiently associated with the phagosome.

The 7 MYC also accumulated at the phagosome, but, in contrast to 13GFP, did so much more slowly. The 7 MYC was rarely detectable on phagosomes 10 min after the onset of phagocytosis (Fig. 3, D and F), but distinct recruitment was noticeable after 20–30 min (not shown). A pronounced accumulation of 7 MYC in phagosomes was evident at 60 min (Fig. 3, E and F). Thus, unlike 13GFP that associated only transiently with phagosomes, 7 MYC accumulated monotonically throughout the period studied, and no evidence for cycling off the phagosome was observed. We also

![FIGURE 3. Syntaxin 13 and 7 accumulation at the phagosome. RAW cells were transiently transfected with 13GFP (A–C) or 7 MYC (D, E) and allowed to ingest opsonized SRBC for the indicated times. A, Within 2.5 min, 13GFP accumulated on nascent phagosomes (arrows). B, By 5 min, 13GFP could still be seen at nascent phagosomes and was present on most internalized phagosomes (arrowheads). C, By 10 min, 13GFP was absent from internalized phagosomes. D, Only modest association of 7 MYC with phagosomes was noted after 10 min. E, 7 MYC accumulation was observed around all internalized phagosomes after 60 min. The scale bar represents 10 μm. F, Summary of 13GFP, 7 MYC, and LAMP-1 accumulation time course. The percentage of positive phagosomes for each of the markers indicated was counted. Data are means ± SE of 218 phagosomes from 113 cells transfected with 13GFP or 202 phagosomes from 98 cells transfected with 7 MYC from three independent experiments.](http://www.jimmunol.org/doi/10.118/00221717.156.11.3253)
monitored the accumulation of LAMP-1 as a marker of phagosome maturation and, as seen in Fig. 3F, LAMP-1 accumulated with the same time course as 7 MYC.

**Effect of truncated (cytosolic) syntaxin 13 on phagosome formation and maturation**

To determine whether the accumulation of syntaxins 13 and 7 at the phagosome had functional significance to phagosomal maturation, we set out to inhibit their function by using a dominant-negative approach. Earlier studies showed that the introduction into cells of the cytoplasmic portions of SNAREs can exert an inhibitory effect on the function of the endogenous, full-length proteins (19–21). It is thought that these soluble fragments are inhibitory because they can form stable complexes with endogenous cognate SNARE proteins, not having to overcome the energy barrier caused by the repulsion of the two lipid bilayers. Such fragments cannot trigger fusion because they lack the required membrane anchor and therefore negatively compete with the endogenous SNAREs.

Effective dominant-negative action of the cytosolic fragments requires high levels of ectopic expression. RAW 264.7 cells were cotransfected with the cytoplasmic portion of syntaxin 13 (13ΔTM) and with EGFP (10:1 ratio of the CNDs). The fluorescence of the latter was used to identify the transiently transfected cells. To confirm that 13ΔTM was active in inhibiting syntaxin 13 function, we examined the ability of transfected COS-2A to internalize fluorescently labeled transferrin, because syntaxin 13 is implicated in endosome-endosome fusion events (16). Unfortunately, the moderate expression levels achieved following transfection of RAW 264.7 cells did not lead to dominant-negative effects. High expression levels can be best achieved by transfecting plasmids with the SV40 origin of replication in cells expressing the large T Ag, such as the monkey COS cells. However, COS cells are not normally phagocytic, because they lack opsonin receptors. To circumvent this problem, we used COS-2A cells, which are a stable line of COS-1 cells that express the FcγRIIA. These cells have been fully characterized for their ability to support phagocytosis in a manner that closely resembles the behavior of professional phagocytes (4, 22, 23). Expression of 13ΔTM in COS-2A cells resulted in a decrease in the uptake of transferrin (Fig. 4A). Furthermore, the transferrin that was internalized by the transfected cells did not show the perinuclear concentration typical of transferrin labeling in normal cells, which can be readily seen in the adjacent nontransfected cell (Fig. 4A).

To determine whether 13ΔTM had any effect on phagocytosis, transfected cells were incubated in the presence of latex beads opsonized with human IgG. The location of beads could be observed by differential interference contrast microscopy, and the beads that were not internalized could be identified by staining nonpermeabilized cells with Abs specific for human IgG (Fig. 4B, left panels). By counting the number of internalized beads in the transfected cells compared with cells transfected with EGFP alone, we found that 13ΔTM had no effect on particle internalization (Fig. 4C, left histogram). To determine whether 13ΔTM altered the number of phagosomes, cells that had undergone phagocytosis for 60 min were fixed, permeabilized, and stained with Ab to LAMP-1. Phagosomes were then scored for the presence (Fig. 4B, arrows) or absence (Fig. 4B, arrowheads) of LAMP-1 to determine the number of phagosomes positive for this lysosomal protein. As can be seen in Fig. 4C (right histogram), 13ΔTM significantly reduced the number of LAMP-1-positive phagosomes to ~70% of normal.

**Effect of truncated (cytosolic) syntaxin 7 on phagosome formation and maturation**

Similar experiments were then performed to analyze the role of syntaxin 7 by overexpression of the cytosolic portion of this protein (called 7ΔTM). Overexpression of 7ΔTM altered the distribution of cellular LAMP-1 (Fig. 5A). This is most likely due to the inhibitory effect of 7ΔTM on the function of endogenous syntaxin 7 in the traffic of vesicles from the late endosome to the lysosome.

**FIGURE 4.** Dominant-negative syntaxin 13 (13ΔTM) inhibits phagosome maturation. A, Transferrin uptake is inhibited by 13ΔTM. COS-2A cells were transiently transfected with 13ΔTM and EGFP for 24 h before transferrin uptake assays were performed. GFP-positive cells (inset) took up less tetramethylrhodamine isothiocyanate-transferrin than their untransfected neighbors. The scale bar represents 10 μm. B, Phagocytosis is unaffected in 13ΔTM-transfected cells. COS-2A cells transiently transfected with 13ΔTM and EGFP were incubated in the presence of opsonized latex beads for 60 min before fixation. Differential interference contrast image (upper left panel) reveals the location of the beads. External beads were identified by incubation with anti-human Abs before permeabilization (middle left panel). The 13ΔTM-expressing cells were identified by GFP expression (bottom left panel). Cells were then permeabilized and stained for LAMP-1 (main panel in B). Both LAMP-1-positive (arrows) and LAMP-1-negative (arrowheads) phagosomes could be seen within transfected cells. LAMP-1 signals were typically stronger in untransfected vici-

nal cells. C, Quantification of the effect of 13ΔTM on the phagocytic index (average number of beads ingested/100 cells; left ordinate) and on LAMP-1 acquisition by the phagosomes (index reflects the average number of LAMP-1-positive phagosomes/100 cells; right ordinate). Control samples were transfected with EGFP alone. Data are means ± SE of a total of 84 phagosomes from 43 cells transfected with GFP and 165 phagosomes from 70 cells transfected with 13ΔTM from three independent experiments each.
untransfected cells. B, Phagocytosis is unaffected in 7Δ TM-transfected cells. COS-2A cells transiently transfected with 7Δ TM and EGFP for 24 h were fixed and stained with Ab to LAMP-1. GFP-positive cells (inset and outlined in panel) dis-
limiting the extension of pseudopods. In contrast, inhibition of syntaxin 13 function appeared to have no detectable effect on phagocytosis per se, but interfered with the subsequent steps required for maturation of the phagosome. Interestingly, coexpression of 13/H9004 with 7 MYC is not overexpressed (Fig. 4), a more complete blockade of syntaxin 7 occurs, limiting LAMP-1 arrival. Future studies will be aimed at addressing these two possibilities.

Based on the SNARE model, it is possible to predict that VAMP-3 functions as the vesicular SNARE required for the fusion of vesicles from the recycling endosome with the target membrane. In this case, the target membrane is the region of the plasma membrane defined by the activation of the FcR engaged by the opsonized particle. Previous studies had shown that phagosomes also contained syntaxins 2, 3, and 4 (30), and these are plasma membrane-localized forms of syntaxin that are thought to be cognate partners for VAMP-2 and VAMP-3 (31). It is likely, therefore, that VAMP-3 functions in conjunction with plasma membrane resident syntaxin isoforms to provide the fusion events needed to form the nascent phagosome. Although syntaxin 13 delivery to the phagosome is not required for phagosome formation, it may serve to change the membrane milieu by making the nascent phagosome competent for fusion with endosomes at the early stages of phagosome maturation. Each step in the endocytic pathway most likely requires distinct sets of syntaxin proteins to receive the appropriate transport vesicles. For phagosome maturation to mimic the stepwise progression through the endocytic pathway would therefore require the removal of the existing syntaxins with replacement by the syntaxin responsible for the next step. Based on our observations, we would hypothesize that syntaxin 13 serves to facilitate early endosome fusion events, while syntaxin 7 would be required to permit the fusion of vesicles from the late endosome/lysosome compartments. This would be consistent with in vitro studies showing that young phagosomes fuse efficiently with endosomes, while old phagosomes can fuse best with lysosomes.

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