

10-1-2009

Intracellular bacteria encode inhibitory SNARE-like proteins.

Fabienne Paumet

Thomas Jefferson University, Department of Microbiology and Immunology, Philadelphia, PA

Jordan Wesolowski

Thomas Jefferson University, Department of Microbiology and Immunology, Philadelphia, PA

Alejandro Garcia-Diaz

Department of Cell Biology, Yale University, New Haven, CT


Cedric Delevoye

Institut Curie, Structure et Compartiments Membranaires, CNRS-UMR144, Paris, France

Nathalie Aulner

Institut Pasteur, Imagopole, Batiment Monod, Paris, France

Follow this and additional works at: <https://jdc.jefferson.edu/mifp>

 Part of the [Medical Cell Biology Commons](#), [Medical Genetics Commons](#), [Medical Immunology Commons](#), and the [Medical Microbiology Commons](#)
See next page for additional authors

[Let us know how access to this document benefits you](#)

Recommended Citation

Paumet, Fabienne; Wesolowski, Jordan; Garcia-Diaz, Alejandro; Delevoye, Cedric; Aulner, Nathalie; Shuman, Howard A; Subtil, Agathe; and Rothman, James E, "Intracellular bacteria encode inhibitory SNARE-like proteins." (2009). *Department of Microbiology and Immunology Faculty Papers*. Paper 20. <https://jdc.jefferson.edu/mifp/20>

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Microbiology and Immunology Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Authors

Fabienne Paumet, Jordan Wesolowski, Alejandro Garcia-Diaz, Cedric Delevoye, Nathalie Aulner, Howard A Shuman, Agathe Subtil, and James E Rothman

Intracellular Bacteria Encode Inhibitory SNARE-Like Proteins

Fabienne Paumet^{1*}, Jordan Wesolowski¹, Alejandro Garcia-Diaz², Cedric Delevoye³, Nathalie Aulner⁴, Howard A. Shuman⁵, Agathe Subtil⁶, James E. Rothman²

1 Thomas Jefferson University, Department of Microbiology and Immunology, Philadelphia, Pennsylvania, United States of America, **2** Department of Cell Biology, Yale University, New Haven, Connecticut, United States of America, **3** Institut Curie, Structure et Compartiments Membranaires, CNRS-UMR144, Paris, France, **4** Institut Pasteur, Imagopole, Batiment Monod, Paris, France, **5** Department of Microbiology, Columbia University Medical Center, New York, New York, United States of America, **6** Institut Pasteur, Unité de Biologie des Interactions Cellulaires, CNRS-URA 2582, Paris, France

Abstract

Pathogens use diverse molecular machines to penetrate host cells and manipulate intracellular vesicular trafficking. Viruses employ glycoproteins, functionally and structurally similar to the SNARE proteins, to induce eukaryotic membrane fusion. Intracellular pathogens, on the other hand, need to *block* fusion of their infectious phagosomes with various endocytic compartments to escape from the degradative pathway. The molecular details concerning the mechanisms underlying this process are lacking. Using both an *in vitro* liposome fusion assay and a cellular assay, we showed that SNARE-like bacterial proteins block membrane fusion in eukaryotic cells by directly inhibiting SNARE-mediated membrane fusion. More specifically, we showed that IncA and IcmG/DotF, two SNARE-like proteins respectively expressed by *Chlamydia* and *Legionella*, inhibit the endocytic SNARE machinery. Furthermore, we identified that the SNARE-like motif present in these bacterial proteins encodes the inhibitory function. This finding suggests that SNARE-like motifs are capable of specifically manipulating membrane fusion in a wide variety of biological environments. Ultimately, this motif may have been selected during evolution because it is an efficient structural motif for modifying eukaryotic membrane fusion and thus contribute to pathogen survival.

Citation: Paumet F, Wesolowski J, Garcia-Diaz A, Delevoye C, Aulner N, et al. (2009) Intracellular Bacteria Encode Inhibitory SNARE-Like Proteins. PLoS ONE 4(10): e7375. doi:10.1371/journal.pone.0007375

Editor: Raphael H. Valdivia, Duke University Medical Center, United States of America

Received: July 31, 2009; **Accepted:** September 15, 2009; **Published:** October 12, 2009

Copyright: © 2009 Paumet et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by National Institutes of Health Grants AI23549 (H.A.S.), AI064481 (H.A.S.), and grants to J.E.R. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: fabienne.paumet@jefferson.edu

Introduction

In eukaryotic cells, intracellular membrane fusion events are mediated by members of the SNARE protein family. SNAREs are conserved in all eukaryotes and are present on the surface of all secretory compartments [1,2,3]. During membrane fusion, t-SNAREs present on target organelles assemble into a four-helix bundle with the v-SNAREs present on vesicles. This event brings the membranes in which they are embedded into close apposition and drives bilayer fusion [4,5,6,7]. The SNARE residues indispensable for membrane fusion form the “SNARE motif” [6], a 60 amino-acid sequence composed of coiled-coil heptad repeats [Table 1 and [8]]. Similar structural motifs are used for the same purpose by viruses, highlighting the general role of coiled coil sequences in manipulating membrane fusion [9,10]. Here we investigated whether this particular motif is also utilized by bacteria to influence eukaryotic membrane fusion.

Intracellular bacteria such as *Salmonella*, *Mycobacterium*, *Legionella* or *Chlamydia* must manipulate membrane fusion of the host cells they inhabit in order to escape lysosomal fusion [11,12]. While intracellular, these bacteria modify their infectious phagosomes, also called inclusions or vacuoles, by expressing their own proteins to the surface [13]. As a result, the infectious phagosomes become protected against fusion with endocytic compartments [14,15].

Although the precise mechanism is unclear, it is likely that the bacterial proteins expressed on the surface of these infectious phagosomes are responsible for blocking fusion with the endocytic compartments [16,17]. Interestingly, over the past few years a growing number of SNARE-like proteins have been identified notably in *Chlamydia* and *Legionella* [18,19,20], two intracellular bacteria responsible for human diseases. For instance, IncA, a protein expressed by *Chlamydia* on the surface of the infectious vacuole displays two SNARE-like motifs [19,21]. IncA interacts directly with mammalian SNAREs [21] and IncA expressed by *Chlamydia trachomatis*, CtrIncA, has been implicated in homotypic membrane fusion [22,23]. Formation of *Chlamydia* inclusions by homotypic fusion is an event specifically occurring during *C. trachomatis* infection. Interestingly, most *Chlamydia* strains express IncA, yet not all strains have the capacity to undergo homotypic fusion, suggesting that IncA likely plays additional roles.

Expressed by *Legionella pneumophila*, IcmG/DotF only displays one SNARE-like motif. The precise function of IcmG/DotF is still unclear, although mutants are rapidly trafficked to, and degraded within lysosomal compartments [24]. Using these bacterial SNARE-like proteins as our models, we tested their function on SNARE-mediated membrane fusion. We discovered a novel inhibitory function of these proteins and characterized the molecular mechanism they use to block host membrane fusion.

Table 1. SNARE motifs alignment.

		...*...*...*...*...*...*...*...*...*...*...*...*...*...*...*...*	
hSNAP25-Nterm	19	DQLADESLESTRRLQLVEESKDAGIRTLVMLDEQGEQLERIEEGMDQINKDMKEAEKNL	
hSNAP25-Cterm	140	DARENEMDENLEQVSGIIGNLRHMALDMGNEIDTQNRQIDRIMEKADSNTKTRIDEANQRA	
hSNAP23-Cterm	146	DAREDEMEENLTQVGSILGNLKDMLNIGNEIDAQNPQIKRITDKADTNRDRIDIANARA	
Sec9p-Cterm	588	DEMELEIDRNLDQIQQVSNRLKMKALTTGKELDSQQKRLNNIEESTDDLDINLHMNTNRL	
hStx1a	192	LSEIETRHSIIEIKLENSIRELHDMFMDMAMLVESQGEMIDRIEYNVEHAVDYVERAVSDT	
hStx4	200	LNEISARHSEIQQLERSIRELHDIFFLATEVEMQGEVINRIEKNILSSADYVERGQEHV	
Sso1p	190	LAEVQARHQELKLEKSMALTLQLFNDMEELVIEQOENVVDIDKNVEDAQLDVEQGVGHT	Q-SNAREs
hStx5	209	DSYIQSRADTMQNIESTIVELGSIFFQQLAHMVKEQEETIQRIDENVLGAQLDVEAAHSEI	
Sed5p	249	NVYLQERNRAVETIESTIQEVGNLFQQLASMVQEQGEVIQRIDANVDDIDLNISGAQREL	
Vam3p	190	TIHQERSQQIGRIHTAVQEVNAIFHQGLSLVKEQGEQVTTIDENISHLHDNMQNANKQL	
hStx7	165	LRLIHERESSIRQLEADIMDINEIFKDLGMMIHEQGDVIDSIEANVENAEVHVQANQQL	
Pep12p	195	QNLIEQRDQEISNIERGITELENEVFKDLGSVQQQGVLVNDIEANIYTTSDNTQLASDEL	
Tlg2p	244	EAYLREERDEITQLARGVLEVSTIFREMQDLVVDQGTIVDRIDYNLENTVVELKSADKEL	
Stx6	163	QLIVEQQDEQLELVSGSIGVLNKMSQRIGGELEEQAVMDDFSHELESTQSRLDNVMKKL	
Tlg1p	132	EQMLREQDVHLDGIHKTMLNLIQAQTMGDELENGQQLLDNMDEGMGVDVKNLARGRRQL	
Vam7p	250	MQMVRDQEQELVALHRIQAQRGLALEMNEELQTNELLETALEDDVDNTGRRQLQIANKKA	
Vtilp	124	HAILQKSGDRLKDASRIANETEGIGSQIMMDLRSQRETLNARQTLFQADSVDKSIKTL	
IcmG/DotF	146	GEQINAVNNNIKNLNAQIVNLNQIIGNMSNQIARQSEVINVMARTTPKVVVKVSRPIVQ	
CtrInca-Nterm	92	YQDLQREVGSLKEINFMLSVLQKEFLHLSKEFATTSKDLSAVSDQDFYSCLQGFRDNYKGF	SNARE-
CtrInca-Cterm	210	TVVIEELKTIKIRDSLDEIGQLSLSKTLTSQIALQRKESDLSQSIRETLLSSPRKSASPS	like
CcaInca-Nterm	126	VRHMQQIQQFGEENTRLHTAVENLKAVNVELSEQINQLKQLHTRLSDFGDRLEANTGDF	proteins
CcaInca-Cterm	233	MSSVTELRTNLNLKELITENKTVIEQLKADAQLREEQVRFLEKQKQEEACSTLSHSI	
hSyb1	25	PPNMTS.NRRLQQTQAQVVEVVDIIRVNVDKVLERDQKLELDDRADALQAGASQFESSA	
hSyb2	22	PPNLTS.NRRLQQTQAQVVEVVDIMRVNVDKVLERDQKLELDDRADALQAGASQFETSA	
hSyb3	6	TAATGS.NRRLQQTQNVQVVEVVDIMRVNVDKVLERDQKLELDDRADALQAGASQFETSA	
hVAMP8	2	EASEGGGNDVRNLQSEVEGVKNIMTQNVIRILARGENLEHLRNKTELEATSEHFKTTS	R-SNAREs
Snc1p	20	PQNVQS.KSRTAELQAEIDDTVGIMRDNINKVAERGERLTSIEDKADNLAVSAQGFKRGA	
Nyv1p	157	NGQNTI.SDIGDATEDQIKVDVIQIMNDNIDKFLERQERVSLVLDKTSQLNSSSNKFRKKA	
Sec22p	122	SYSDKKVQDNLQNLQELVGVKQIMSKNIEDLLYRGDSLKMSDMSSSLKETSKRYRKA	
mSec22b	126	YIDSRA.RRNLSINTELQDVQRIMVANIEEVLQRGEALSALDSKANNLSSLSKKYRQDA	

SNARE motifs from yeast and mammals were aligned with CtrInca-N and C-term, CcaInca-N and C-term and IcmG/DotF's SNARE-like motifs (grey). The amino acids indicating the layers in the heptad repeat are highlighted in bold (asterisk). Notice the conserved glutamine and arginine residues in the central 'd'-position of the heptad repeat, which constitute the zero layer. Stx = syntaxin. N-term and C-term refer to the N-terminal and C-terminal coiled-coil domain, respectively.

doi:10.1371/journal.pone.0007375.t001

Results and Discussion

Intracellular bacteria primarily protect their vacuoles against endocytic fusion [12,25], which is mediated by the association of the v-SNARE VAMP8 with the endocytic t-SNARE composed of Syntaxin 7, Syntaxin 8 and Vtilb [26,27].

Two distinct complementary mechanisms have been suggested concerning chlamydial avoidance of lysosomal fusion: 1) during the first ~8 hours of infection, the protection of the vacuole appears to be independent of *Chlamydia* protein synthesis [28]. Rather, structural components of the *Chlamydia* cell wall seems to be involved in this activity [29]. 2) Later however, at a time that coincides with IncA expression [30], an active modification of the inclusion membrane takes place to sustain the protection of the inclusion. In light of these evidences, we started to investigate the role of IncA in the protection of the Chlamydia inclusion.

Previously, IncA has been shown to co-precipitate with the endocytic SNAREs when expressed in cells [21]. Using an *in vitro* liposome fusion assay [4,6], we now tested both CtrInca and

CcaInca expressed respectively by *C. trachomatis* and *C. caviae*, for their functional effect on endocytic SNARE-mediated membrane fusion (see Table 2 for a description of all the SNARE proteins studied here). To do so, we reconstituted the t-SNARE [Syntaxin7/Syntaxin8/Vtilb] and the v-SNARE [VAMP8] with or without IncA into acceptor and donor liposomes, respectively. Donor liposomes contain the FRET pair Rhodamine-PE [N-(lissamine rhodamine B sulfonyl) phosphatidyl ethanolamine] and NBD-PE [N-(7-nitro-2,1,3-benzoxadiazole-4-yl) phosphatidyl ethanolamine]. Liposome fusion results in lipid mixing of donor and acceptor liposomes. As the distance between NBD and rhodamine increases, the resonance energy transfer and the quenching of NBD are reduced. Fusion becomes detectable as an increased NBD fluorescence at 538nm [4,6]. After mixing different combinations of t- and v- liposomes +/- IncA, liposome fusion was allowed to proceed at 37°C for two hours. As shown in Fig. 1, CtrInca strongly inhibits endocytic SNARE-mediated fusion. CtrInca blocks membrane fusion whether present in v-SNARE (~70% inhibition) or in t-SNARE (~37% inhibition) liposomes

Table 2. SNARE proteins description.

SNARE investigated	Category	Location
Syntaxin7	t-SNARE	Late endosome/lysosome
Syntaxin8	t-SNARE	Late endosome/lysosome
Vti1b	t-SNARE	Late endosome/lysosome
VAMP8	v-SNARE	Late endosome/lysosome (mast cell secretory granules)
Syntaxin2	t-SNARE	Plasma membrane
Syntaxin3	t-SNARE	Plasma membrane
Syntaxin4	t-SNARE	Plasma membrane
SNAP23	t-SNARE	Plasma membrane
VAMP2	v-SNARE	Secretory vesicle

The SNAREs involved in endocytosis are Syntaxin 7, Syntaxin 8, Vti1b and VAMP8, while the SNAREs involved in exocytosis are Syntaxin 2, Syntaxin 3, Syntaxin 4, SNAP23 and VAMP2.

doi:10.1371/journal.pone.0007375.t002

(Fig. 1B and 1C). We observed that the inhibitory effect of *CtrIncA* correlates with its concentration in the liposomes (Fig. 1C and 1D). Similarly, *CcaIncA* inhibits endocytic SNARE-mediated fusion whether present on t-SNARE (~40% inhibition) or v-SNARE liposomes (~50% inhibition) (Fig. 1G and 1F), confirming the inhibitory role of IncA proteins. When we compared both the effects of IncA and IcmG/DotF, a SNARE-like protein (Fig. 1H) expressed by *Legionella pneumophila* [Table 1 and [18]], we also observed inhibition of SNARE-mediated membrane fusion (Fig. 1). Interestingly, IcmG/DotF has no effect when present on the t-SNARE side (Fig. 1J), but only interferes with the v-SNARE (Fig. 1I). Although both IncA and IcmG/DotF have a common inhibitory function on membrane fusion, it appears that bacterial SNARE-like proteins display different levels of efficiency. Most likely, other SNARE-like proteins, such as LegC3, play a major role in protecting *Legionella*'s vacuole [31].

Although *CtrIncA* has been previously implicated in homotypic membrane fusion [22,23], we did not observe any fusion events between *CtrIncA*-containing liposomes (data not shown). Perhaps *CtrIncA* requires post-translational modifications such as phosphorylation, to become fusogenic [32]. Consistent with this possibility, IncA has multiple phosphorylation sites that become phosphorylated by host cells during infection [19,32]. Alternatively, additional proteins from either *Chlamydia* or the host cell might be necessary in combination with *CtrIncA* to promote fusion. Interestingly, some non-fusogenic strains do express a normal IncA protein on the inclusion membrane, supporting the possibility that other elements of the fusion machinery are missing in these strains [33].

We propose that *CtrIncA* could function as a switch to regulate the maturation of the inclusion. During the infectious cycle of *C. trachomatis*, each newly synthesized *CtrIncA* would first bind every resident SNARE on the inclusion, until all are blocked. As a consequence, SNARE-mediated fusion of the inclusion would be totally inhibited. As *CtrIncA* continues to accumulate, excess *CtrIncA* would then be available for further modification by the host cell (phosphorylation) and/or for binding additional proteins. *CtrIncA* would become active for fusion and inclusions could then undergo homotypic fusion.

Next, we determined whether the inhibitory function was encoded into the SNARE-like motif. Since IcmG/DotF has a limited inhibitory effect, we concentrated our efforts on IncA. IncA possesses two SNARE-like motifs [21] (Table 1, Fig. 1A). We

focused our attention on the N-terminal motif due to its presence next to the trans-membrane domain mimicking the eukaryotic SNARE configuration. This makes it ideally located to interact directly with eukaryotic SNARE motifs. Furthermore, this motif has previously been shown to be compatible with the formation of a stable complex with SNARE proteins [19]. To determine whether the N-terminal SNARE-like motif has an inhibitory activity, truncated forms of *CtrIncA* were generated and their effects on endocytic SNARE-mediated fusion were examined. *CtrIncA* mutant containing only the N-terminal SNARE-like motif (*CtrIncA*₁₋₁₄₁) inhibited endocytic SNARE-mediated fusion in a dose-dependent manner similar to the full-length protein (Fig. 2A). Next, we delineated the minimal IncA sequence necessary to retain the inhibitory function. As shown on Fig. 2B and Fig. 2C, *CtrIncA*₁₋₁₃₀ still displays a significant inhibitory effect (~15%, $p = 0.028$) when present on either t- or v-SNARE membrane. On the contrary, *CtrIncA*₁₋₁₂₀, which contains only half of the SNARE-like motif, completely lost its ability to inhibit endocytic SNARE-mediated membrane fusion (Fig. 2D, 2E, $p = 0.42$). The outcome was similar regardless of whether this truncated form of IncA was reconstituted into t-SNARE or v-SNARE liposomes. This suggests that the *CtrIncA* N-terminal SNARE-like motif requires a SNARE-like motif of at least ~23 amino acids in order to exert an effective inhibitory activity and confirm the role of this motif in blocking membrane fusion. Although the function of the C-terminal domain remains to be determined, we cannot exclude its role in reinforcing the inhibitory effect of IncA.

Since *Chlamydia* inclusion membrane is derived from the plasma membrane, we then decided to test the effect of both *CtrIncA* and *CcaIncA* on the plasma membrane resident exocytic t-SNAREs (Table 2). As shown in Fig. 3A, *CtrIncA* has no inhibitory effect on any of the exocytic complexes tested, regardless of its concentration, suggesting that *CtrIncA* is specific for the endocytic SNAREs. *CcaIncA*, on the other hand, exerts a significant inhibitory effect on [Syn2/SNAP23], [Syn3/SNAP23] and [Syn4/SNAP23] fusion (Fig. 3B), demonstrating that *CcaIncA* has a broader inhibitory effect. These results further show that bacterial SNARE-like proteins display different levels of specificity. One might imagine that the capacity to inhibit a large range of membrane fusion events could potentially increase the number of hosts that intracellular bacteria could infect. For example, *C. caviae* has been detected in a wide range of hosts [34]. Alternatively, blocking a multitude of vesicular trafficking in the cells could impact the long-term outcome of an infection. In particular it would be interesting to correlate the level of SNARE-like protein inhibition with the capacity of certain bacteria to induce chronic diseases.

To confirm SNARE-like proteins inhibitory function in a more physiological environment, we tested IncA's role *in vivo* in mammalian cells that can potentially host infection. In order to obtain quantitative results, we chose the RBL-2H3 mast cell line as our model. Mast cells display a large number of endocytic compartments, including their secretory granules, which are secretory lysosomes [35]. During stimulation, the endocytic v-SNAREs VAMP8 present on the secretory lysosomes bind the exocytic t-SNAREs [Syntaxin 4/SNAP23] present on the plasma membrane to mediate exocytosis [36,37]. If IncA interferes with SNAREs when present in RBL-2H3 mast cells, as it does in the liposomes, we should observe an inhibition of the secretory pathway. Because *CcaIncA* full-length protein was toxic for the cells, RBL-2H3 were transfected with myc-*CcaIncA*₁₋₂₂₀, a truncated form of *CcaIncA* still containing its SNARE-like N-terminal domain [19], and therefore still inhibitory (see Fig. 2). Myc-*CcaIncA*₁₋₂₂₀ was cloned together with GFP into an IRES vector to simultaneously express a transfection marker (40%

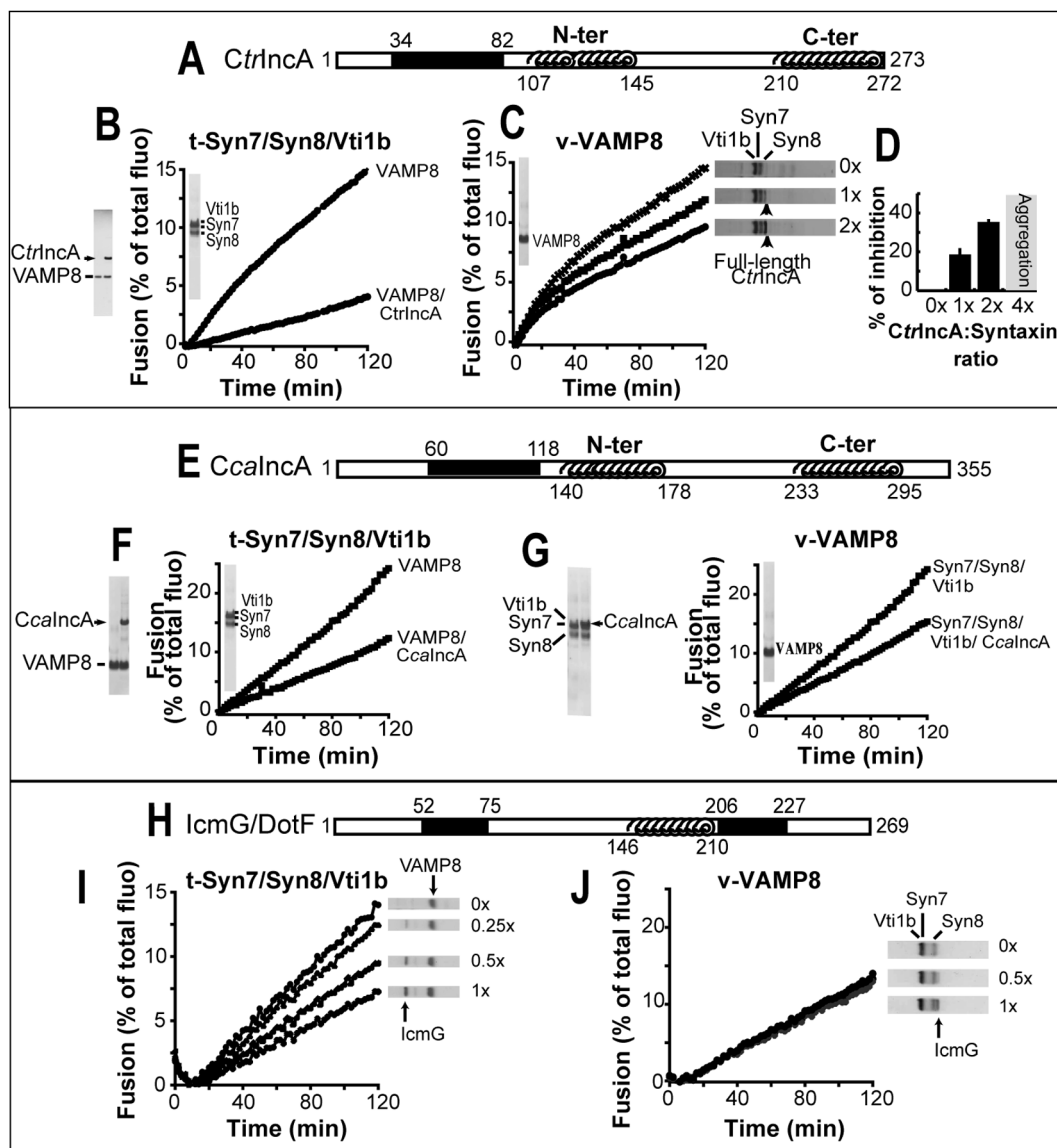


Figure 1. SNARE-like bacterial proteins inhibit endocytic SNARE-mediated membrane fusion. **A-** *CtrlncA* encodes a transmembrane domain (aa34–82), a N-terminal (N-ter: aa107–145) and a C-terminal (C-ter: aa210–272) SNARE-like motif. **B-** VAMP8 was reconstituted with and without *CtrlncA* into donor liposomes (coomassie gel), and incubated with t-SNARE liposomes containing [Syn7/Syn8/Vti1b]. Fusion decreases in presence of *CtrlncA* (70% inhibition). **C-** t-[Syn7/Syn8/Vti1b] was reconstituted with different concentration of *CtrlncA* into acceptor liposomes. Fusion shows a concentration dependency for *CtrlncA* inhibition. **D-** The percentage of inhibition with the standard deviation is plotted (n = 3). For each experiment, results were normalized based on the fusion rate obtained after 2 hrs with the endocytic complex w/o *CtrlncA* (0x). We observed 20% inhibition for a *CtrlncA*:Syn7 estimated ratio of 1:1 (1x). The inhibition rate increased to 35% for an estimated ratio of 2:1 (2x). **E-** *CcalncA* encodes a transmembrane domain (aa60–118), a N-terminal (N-ter: aa140–178) and a C-terminal (C-ter: aa233–295) SNARE-like motif. **F-** We observed 50% inhibition when *CcalncA* was reconstituted on the v-SNARE side with VAMP8. **G-** We observed 40% inhibition when *CcalncA* was reconstituted with [Syn7/Syn8/Vti1b] into acceptor liposomes. **H-** *IcmG/DotF* displays two hydrophobic regions (aa52–75 and 206–227), and a SNARE-like motif (aa146–210). **I-** Endocytic fusion is reduced when *IcmG/DotF* is present in v-SNARE liposomes and the inhibition rate correlates with *IcmG/DotF* concentration, reaching 50% of inhibition for an estimated ratio *IcmG*:VAMP8 of 1:2. **J-** *IcmG/DotF* does not interfere with membrane fusion when present in t-SNARE liposomes. All graphs are representative of at least 3 independent experiments.
doi:10.1371/journal.pone.0007375.g001

average transfection efficiency). Using immunofluorescence, we observed that Myc-*CcalncA*_{1–220} co-localized with lysotracker (Fig. 4A), a marker of the RBL-2H3 secretory lysosomes [38]. This suggests that Myc-*CcalncA*_{1–220} is located on the secretory lysosomes where it can potentially interact with the lysosomal v-SNAREs VAMP8. This is physiologically relevant since VAMP8 is involved in the phagosomal fusion with lysosomes [39]. Therefore, interfering with VAMP8 would protect the phagosomal compartment against degradation. After stimulating transfected

mast cells with both 10^{-7} M Phorbol 12-Myristate 13-Acetate (PMA) and 10^{-6} M ionomycin, we analyzed the release of β -hexosaminidase, a lysosomal enzyme stored inside mast cell secretory lysosomes. Kinetic analyses showed that after 30 min of stimulation, cells transfected with myc-*CcalncA*_{1–220}/GFP secrete significantly less β -hexosaminidase than the GFP control. The level of inhibition at 30 min (23% inhibition) and at 60 min (32% inhibition) is significant ($p < 0.05$ and $p < 0.02$ respectively) compared to GFP transfected cells (Fig. 4B). These data confirm

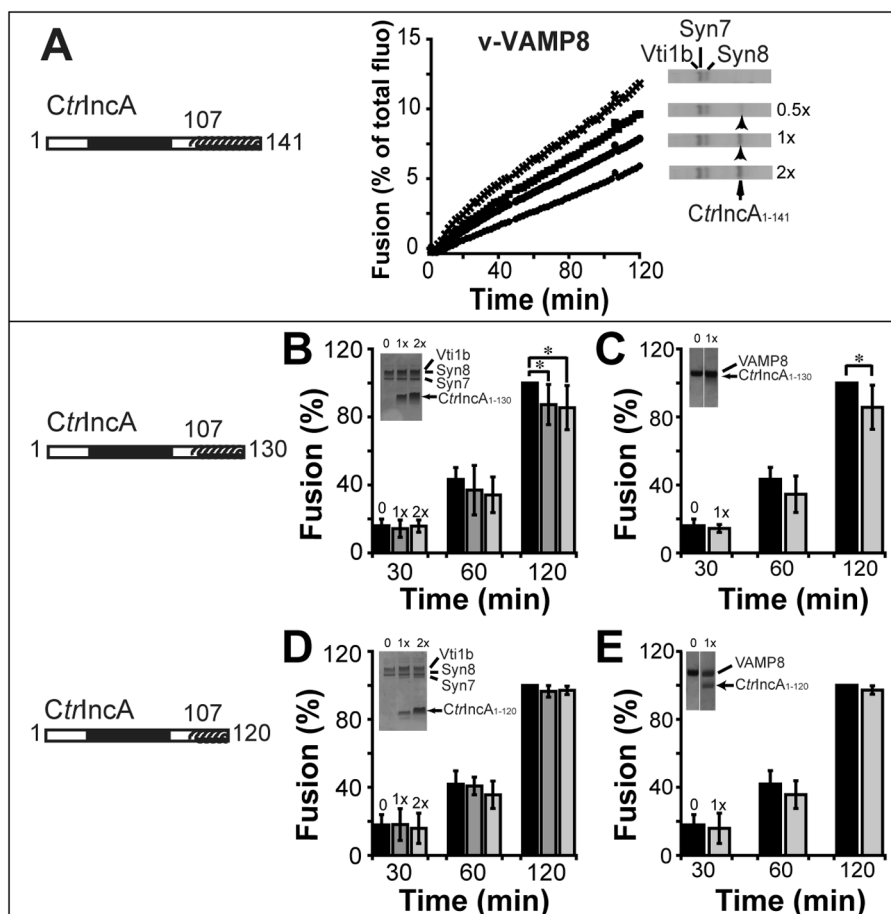


Figure 2. The SNARE-like motif encodes the inhibitory function. **A-** Increasing concentrations of *CtrlncA*₁₋₁₄₁ were reconstituted into endocytic t-SNARE liposomes, and fusion proceeded in presence of VAMP8-liposomes. Fusion is significantly inhibited by the presence of *CtrlncA*₁₋₁₄₁ and is dependent upon its concentration, reaching 55% of inhibition after 2 hrs with an estimated *CtrlncA*₁₋₁₄₁:SNARE ratio of 2:1. This experiment is representative of $n=3$. **B-E-** Two different concentrations of truncated *CtrlncA* (see representative coomassie gels inserted in each graph) were reconstituted into t-SNARE (B,D), and v-SNARE liposomes (C,E). As shown on graphs B and C, *CtrlncA*₁₋₁₃₀ still displays a significant inhibitory effect on the endocytic SNARE-mediated membrane fusion (average of 15% inhibition after 2 hrs, $p=0.022$). On the contrary, *CtrlncA*₁₋₁₂₀ (D,E) completely fails to inhibit endocytic fusion ($p>0.05$). The mean from $n=5$ independent experiments was determined at 30 min, 60 min and 120 min. The standard deviation is shown. One asterisk denotes statistically significant differences ($p<0.05$). For the purpose of comparison, maximal values of fusion obtained for the SNARE complex without *IncA* at 120 min were arbitrarily defined as 100%. doi:10.1371/journal.pone.0007375.g002

the inhibitory effect of *CcaIncA* on t-[Syn4/SNAP23] and v-[VAMP8]-mediated fusion previously observed using the liposome fusion assay (Figs 3 and 1 respectively). Incidentally, this also indicates that the *in vitro* liposome fusion assay is able to accurately predict cellular data. Therefore, this assay represents a unique system by which more bacterial proteins could be screened for their effect on host vesicular trafficking.

Conclusion

The key for survival of intracellular bacteria in host cells is their capacity to manipulate host cellular processes -in particular membrane fusion- to allow the establishment of an intracellular replicative niche. An obvious host machinery to target in order to block membrane fusion is the SNARE machinery. Using *CtrlncA*, *CcaIncA* and *IcmG/DotF* as our models, we demonstrated that SNARE-like bacterial proteins differentially block SNARE-mediated membrane fusion. Furthermore, we showed that this inhibitory function is encoded into their SNARE-like motifs, validating the general function of such a motif for manipulating membrane fusion.

Interestingly, clinical isolates lacking *IncA* present defects in their infectious cycle, and the number of inclusions per cells is significantly decreased [40,41]. This would suggest that the level of protection exerted by *Chlamydia* cell wall during the first 8 hrs [28] is sufficient for small inclusions to develop, but that *IncA* synthesis is necessary for the inclusions to mature further. Alternatively, it could also suggest that additional protective systems, although not as efficient as *IncA*, are in place to insure such an important function. Interestingly, *Chlamydia* was found to express additional SNARE-like bacterial proteins, including CT813, which also interacts with host SNAREs [21]. Although their inhibitory function remains to be confirmed, the redundancy of the SNARE-like protein system would further support its importance. Overlapping layers of protection would insure the survival of *Chlamydia* in case one of the protective systems fails. The differential timing of expression for each of these proteins could also ensure the protection of the vacuole over time [30]. This redundancy would explain the presence of a limited number of *Chlamydia* inclusions during infections with strains naturally lacking *IncA* [41]. Each SNARE-like protein may also be specific for a

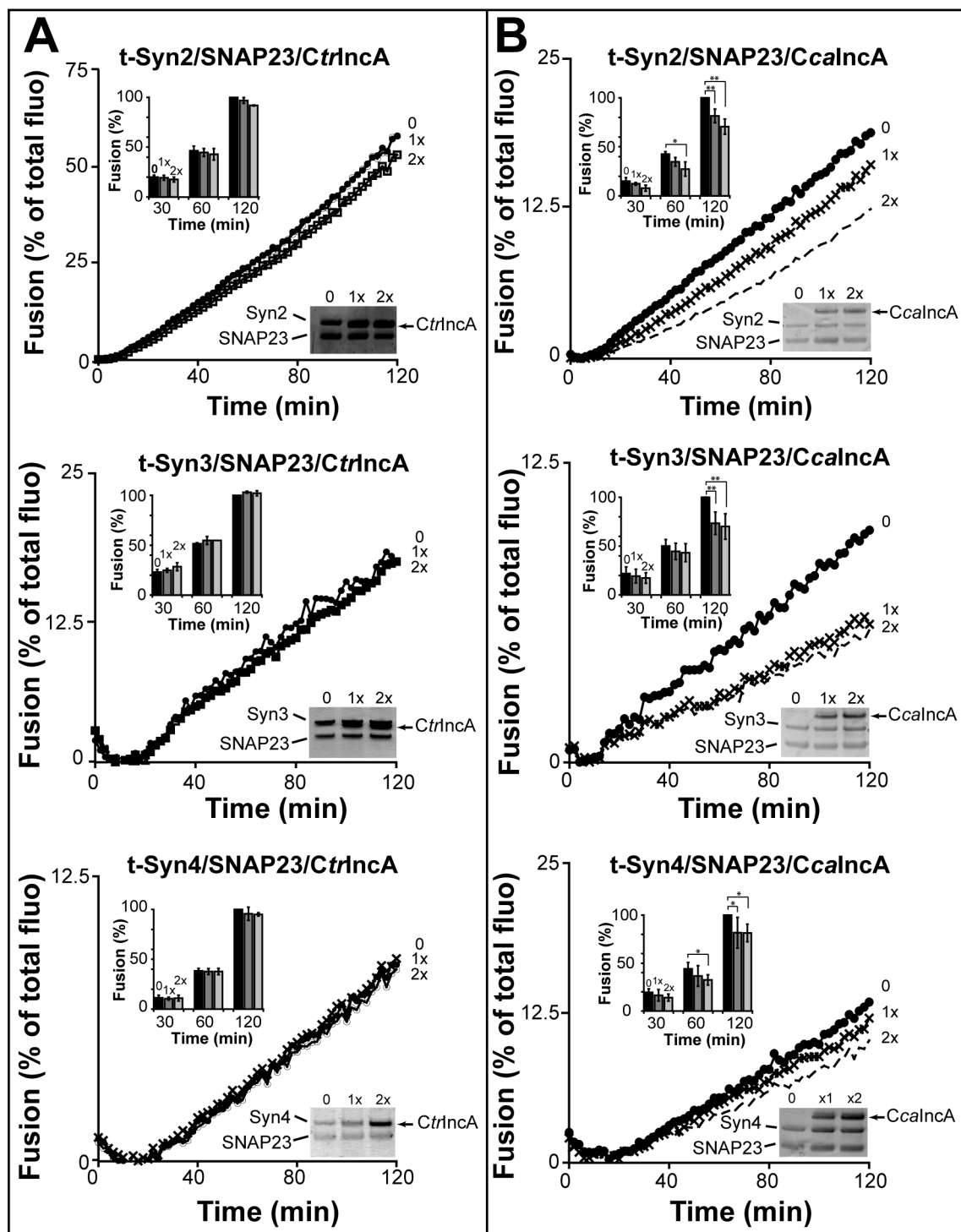


Figure 3. Bacterial SNARE-like proteins display different level of specificity. *CtrlInCA* (A) and *CcalInCA* (B) were reconstituted with the exocytic t-SNARE complexes [Syn2/SNAP23], [Syn3/SNAP23] and [Syn4/SNAP23]. After mixing t-SNARE liposomes (with or without InCA) with VAMP2 liposomes, fusion proceeded. Bar graphs represent the mean from $n = 5$ independent experiments at 30min, 60 min and 120 min for each of the exocytic complex. For the purpose of comparison, maximal values of fusion obtained for the SNARE complex without InCA at 120 min were arbitrarily defined as 100%. The standard deviation is shown. **A-** As shown on the curves and bar graphs, *CtrlInCA* does not affect exocytic fusion regardless of its concentration ($p > 0.05$). **B-** After 2 hrs of fusion, *CcalInCA* significantly inhibits [Syn2/SNAP23]-mediated fusion by 35%, [Syn3/SNAP23]-mediated fusion by 25% and [Syn4/SNAP23]-mediated fusion by 20% ($p = 0.0079$). One and two asterisks denote statistically significant differences with $p < 0.05$, and $p < 0.02$ respectively.

doi:10.1371/journal.pone.0007375.g003

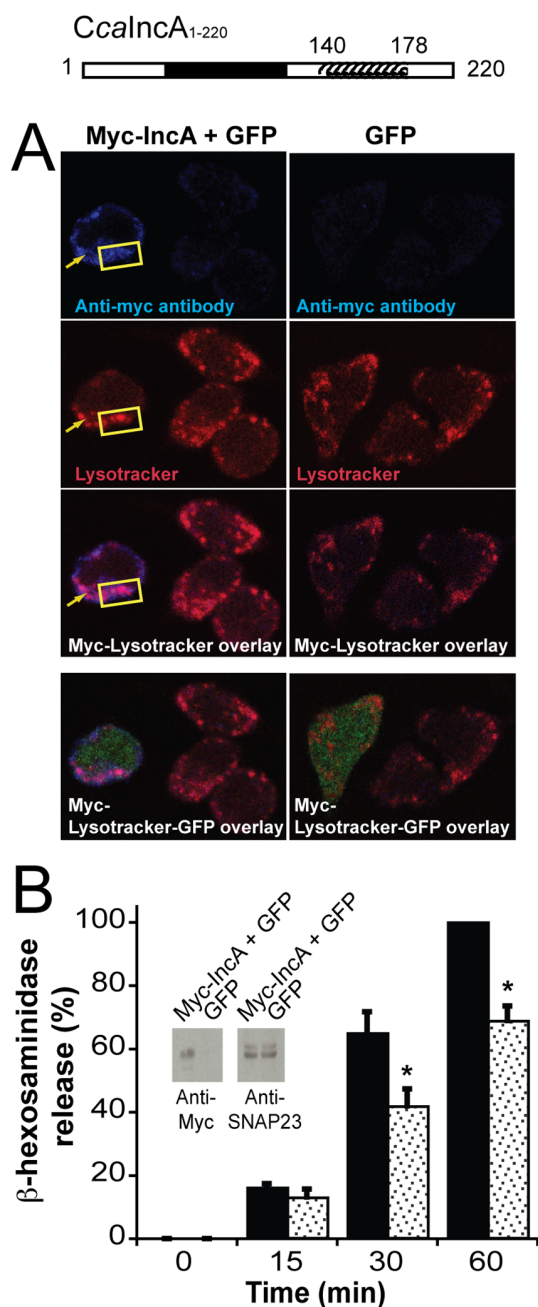


Figure 4. SNARE-like proteins inhibit intracellular fusion in cells. **A-** Resting transfected RBL-2H3 cells were co-labeled with anti-Myc Abs and lysotracker, and viewed by confocal microscopy. Myc-CcalncA₁₋₂₂₀/GFP is on the left, while GFP control is on the right. Colocalized Myc-CcalncA₁₋₂₂₀ and lysotracker compartments are indicated with a yellow box and arrows. **B-**RBL-2H3 cells were transiently transfected with Myc-CcalncA₁₋₂₂₀/GFP or with GFP alone. Total lysates were migrated on SDS-PAGE and probed with Abs directed against Myc. Equivalent amounts of protein in each lane was verified after reprobing the blots with the anti-SNAP23. After stimulation of the transfectants at different time points with 10^{-7} M PMA/ 10^{-6} M ionomycin, the kinetics of degranulation was analyzed using the β -hexosaminidase release assay. The mean of triplicates from five independent experiments was determined. Standard errors are shown. For the purpose of comparison, maximal values of degranulation obtained for GFP-transfected cells at 60 min were arbitrarily defined as 100%. Transfection of Myc-CcalncA₁₋₂₂₀ (Grey bars) reduces mast cells degranulation by 23% at 30 min and 31.8% at 60 min compared with GFP (Dark bars). The asterisks denote statistically significant difference

($p < 0.05$) to GFP transfectants. Note that Myc-CcalncA₁₋₂₂₀/GFP and GFP are not statistically different at 15 min ($p = 0.26$).
doi:10.1371/journal.pone.0007375.g004

different set of host SNAREs, which would increase the protection of the infectious vacuoles against a larger range of membrane fusion events.

It is intriguing to notice that the inhibitory mechanism displayed by bacterial SNARE-like proteins is very similar to the one employed by the eukaryotic inhibitory-SNAREs (i-SNAREs). SNARE-mediated fusion is triggered by four fusogenic subunits and is highly specific [42,43,44,45]. It was shown that the presence of a fifth SNARE on the same compartment could result in an inhibition of fusion (therefore, such a SNARE has been called inhibitory-SNARE). An i-SNARE can substitute for one of the subunits of the functional tetramer leading to the formation of a non-functional tetramer (acting as a pseudo t-SNARE) [46]. In the Golgi, it has been demonstrated that a gradient of i-SNAREs across cisternae blocks SNARE-mediated membrane fusion and is likely used to fine-tune the specificity of membrane fusion [46]. Here we showed that bacterial SNARE-like proteins appear to function in a similar fashion. Similar to i-SNAREs, these bacterial proteins are capable to bind fusogenic SNAREs and inhibit membrane fusion. Altogether, this suggests that coiled-coil SNARE-like motifs may constitute one of the most effective motifs to manipulate membrane fusion and has been incorporated into intracellular bacteria genome as an adaptation to the pressures of survival [47]. Ultimately, one could take advantage of such a recurrence to develop a common therapeutic strategy for targeting a wide array of bacterial SNARE-like proteins and revert the fusion blockage.

Materials and Methods

DNA manipulation and plasmid construction

Standard genetic manipulations were performed throughout. All polymerase chain reaction (PCR) procedures were done with pfu turbo polymerase (Stratagene). All other DNA modifying enzymes were from New England Biolabs. The *E. coli* strain DH5 α (Invitrogen) was used for standard cloning. Plasmid encoding CcalncA₁₂₂ was generated as described [19]. We added a myc tag and cloned CcalncA₁₂₂ into the pIRES2-EGFP vector (Clontech) using the oligonucleotides FO134 GGAATTCATATGACAGTATCCACAGACAACAC and FO135 CGGGATCCTCACAGATCCTCTTCTGAGATGAGTTTTTGTCCAAAGACTGAGCTAATTTCT.

Plasmids encoding Syntaxin 2 (untagged), Syntaxin 3 (untagged), Syntaxin 4 (untagged) and His₆-SNAP23 were kindly provided by Jingshi Shen (Columbia University, New York). Plasmids encoding Syntaxin 7-His₆, Syntaxin 8-His₆, Vti1-His₆ and VAMP8-His₆ were generated as described [45]. Plasmids encoding His₆-CtInca and His₆-CcalncA were generated as described in [19]. Plasmid encoding His₆-IcmG/DotF was generated by PCR using the oligonucleotides FO117 GCGAATTCTCAACTATCTTCTTGACTAAACT and FO118 GGGCATATCCATATGATGGCAGAGCAGCAGATCA. PCR fragments were subsequently ligated into the *EcoRI-NdeI* sites of pET28a. Plasmids encoding His₆-CtInca₁₋₁₄₁, His₆-CtInca₁₋₁₃₀ and His₆-CtInca₁₋₁₂₀ were generated by PCR, respectively using the oligonucleotides FO160 GGGCATATCCATATGACAACGCCTACTCTAATCGTG and FO162 GATGGATCCCTAGTCTTTAGATGTCGTTGCAAAT; FO160 and FO163 GATGATCCCTATAAATGAAGAAATTCCTTTCTG. PCR fragments were subsequently ligated into the *NdeI-BamHI* sites of pET28a.

Protein expression and purification

VAMP8-His₆, Syntaxin8-His₆, Syntaxin7-His₆ and Vti1b-His₆ were expressed as described [45]. Plasma membrane t-SNARE proteins Syntaxin3/His₆-SNAP23, Syntaxin4/His₆-SNAP23, Syntaxin 2/His₆-SNAP23 were co-expressed in BL21 (DE3) star *E. coli* (Invitrogen) and co-purified using the His₆ tag present on SNAP23.

All constructs derived from the bacterial proteins: CcaIncA-His₆, CtrIncA-His₆, His₆-CtrIncA₁₋₁₄₁, His₆-CtrIncA₁₋₁₃₀, His₆-CtrIncA₁₋₁₂₀ and IcmG/DotF-His₆ were expressed in BL21 (DE3) star *E. coli* for 12 hrs at 16°C to allow a proper folding of the protein. All his-tagged proteins were purified using the procedure previously described [44,45,48].

Reconstitution into liposomes

SNARE proteins were reconstituted into proteoliposomes by detergent dilution and isolated on an Accudenz density gradient flotation as previously described [6,49]. To insert bacterial proteins into liposomes, v-SNARE protein and preformed t-SNARE complexes were respectively preincubated with the bacterial protein at different concentration for 4 hrs at 4°C, before being mixed with the lipids, and dialysed for 16 hrs at 4°C.

Liposome fusion assay

Fusion reactions and data analysis were performed as previously described [6,49]. For most fusion assays, the mean from at least 5 independent experiments was determined at 30 min, 60 min and 120 min. For the purpose of comparison, maximal values of fusion obtained for the SNARE complex without IncA at 120 min were arbitrarily defined as 100%. The Mann-Whitney *U* test was used to compare the mean values of maximal fusion at 120 min between SNARE-containing liposomes and SNARE/IncA-containing liposomes. Significance was assumed at *p* values < 0.05.

Cell transfection

The rat mast cell line RBL-2H3 was cultured as described [37]. We used the AMAXA nucleofector technology (AMAXA, Germany) to transiently transfect the RBL-2H3 cells. Briefly, 2 × 10⁶ cells were nucleofected in 100 μl solution V (AMAXA) using 1 μg of pIRES2-EGFP-CcaIncA₁₂₂ vector or pIRES2-EGFP vector (control). The cells were nucleofected using the program T-030. Cells were then plated in complete medium in 96 well plates for subsequent secretory cell assays 12 hrs later. Using these conditions, the efficiency of transfection was routinely in the range of 30 to 40% as determined by immunofluorescence (GFP positive).

Confocal immunofluorescence microscopy

Lysotracker labeling was performed following the manufacturer's instruction. Briefly, cells grown on coverslips were incubated with

lysotracker 1:20,000 for 20 min in complete medium and washed three times. The Myc tag labeling was performed as described [37]. We used the anti-myc antibody (9E10) from Santa Cruz Biotechnology. Cy5-conjugated anti-mouse antibody was from Jackson Laboratories. All data were analyzed using a Leica TCS SP confocal microscope, LEICA CONFOCAL 2.5 software, HCX PL APO 63X oil immersion objective.

Secretory cell assay

Transfectants were plated in 96 well plates in triplicates at ~5 × 10⁵ cells in 100 μl of complete DMEM medium and incubated overnight at 37°C. After 12 hrs, adherent RBL cells were washed twice in prewarmed phenol red free DMEM and stimulated by Phorbol Myristate Acetate (10⁻⁷M)/ionomycin (10⁻⁶M). At different time points (0, 15 min, 30 min 1 hr), 25 μl of supernatant was collected and the granule secretion marker β-hexosaminidase was analyzed using test supernatants within the linear range of the assay [50]. Total cellular content of β-hexosaminidase was determined by lysis of the adherent cells in 0.5% Triton X-100. The absorbance was determined at 410 nm in a micro-titer plate reader. Results were calculated as a percentage of total β-hexosaminidase in cells after correction for spontaneous release in unstimulated cultures. For the purpose of comparison, all data were normalized to the maximal value of β-hexosaminidase release obtained in pIRES2-EGFP transfectants and arbitrarily taken as 100%. The Mann-Whitney *U* test was used to compare the mean values of maximal release between GFP and Myc-CcaIncA₁₋₂₂₀ transfectants. Significance was assumed at *p* values < 0.05.

SDS-PAGE and Western blot analysis

Western blots were performed as described [51]. The anti-myc antibody (9E10) was from Santa Cruz Biotechnology, the anti-SNAP23 antibody from Synaptic System and both were used at 1:500. The secondary antibodies were from Biorad and were used at 1:20,000.

Acknowledgments

We thank Bertha Conyers for technical assistance, as well as Drs. James McNew, Thomas Melia and Peter Antinozzi for their critical comments of the manuscript and helpful discussions. We are also grateful to Gregory Lavieu for his constant interest and helpful comments during the course of this study.

Author Contributions

Conceived and designed the experiments: FP CD AS JER. Performed the experiments: FP JW AGD NA. Analyzed the data: FP JW AGD CD AS JER. Contributed reagents/materials/analysis tools: FP JW AGD CD NA HAS AS. Wrote the paper: FP.

References

- Fasshauer D, Sutton RB, Brunger AT, Jahn R (1998) Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. *Proc Natl Acad Sci U S A* 95: 15781–15786.
- Low S, Chapin S, Wimmer C, Whiteheart S, Kömüves L, et al. (1998) The SNARE machinery is involved in apical plasma membrane trafficking in MDCK cells. *J Cell Biol* 141: 1503–1513.
- Fasshauer D, Eliason W, Brünger A, Jahn R (1998) Identification of a minimal core of the synaptic SNARE complex sufficient for reversible assembly and disassembly. *Biochemistry* 37: 10354–10362.
- Weber T, Zemelman B, McNew J, Westermann B, Gmachl M, et al. (1998) SNAREpins: Minimal machinery for membrane fusion. *Cell* 92: 759–772.
- Nickel W, Weber T, McNew JA, Parlati F, Sollner TH, et al. (1999) Content mixing and membrane integrity during membrane fusion driven by pairing of isolated v-SNAREs and t-SNAREs. *Proc Natl Acad Sci U S A* 96: 12571–12576.
- Parlati F, Weber T, McNew JA, Westermann B, Sollner TH, et al. (1999) Rapid and efficient fusion of phospholipid vesicles by the alpha-helical core of a SNARE complex in the absence of an N-terminal regulatory domain. *Proc Natl Acad Sci U S A* 96: 12565–12570.
- Schuette CG, Hatsuzawa K, Margittai M, Stein A, Riedel D, et al. (2004) Determinants of liposome fusion mediated by synaptic SNARE proteins. *Proc Natl Acad Sci U S A* 101: 2858–2863.
- Weimbs T, Low SH, Chapin SJ, Mostov KE, Bucher P, et al. (1997) A conserved domain is present in different families of vesicular fusion proteins: a new superfamily. *Proc Natl Acad Sci U S A* 94: 3046–3051.
- Skehel JJ, Wiley DC (1998) Coiled coils in both intracellular vesicle and viral membrane fusion. *Cell* 95: 871–874.
- Sollner T (2004) Intracellular and viral membrane fusion: a uniting mechanism. *Curr Opin Cell Biol* 16: 429–435.

11. Fields KA, Hackstadt T (2002) The Chlamydial inclusion: Escape from the endocytic pathway. *Annu Rev Cell Dev Biol* 18: 221–245.
12. Meresse S, Steele-Mortimer O, Moreno E, Desjardins M, Finlay B, et al. (1999) Controlling the maturation of pathogen-containing vacuoles: a matter of life and death. *Nat Cell Biol* 1: E183–188.
13. Rockey DD, Scidmore MA, Bannantine JP, Brown WJ (2002) Proteins in the chlamydial inclusion membrane. *Microbes and Infection* 4: 333–340.
14. Duclos S, Desjardins M (2000) Subversion of a young phagosome: the survival strategies of intracellular pathogens. *Cell Microbiol* 2: 365–377.
15. Dautry-Varsat A, Balañá M, Wyplosz B (2004) Chlamydia-host cell interactions: recent advances on bacterial entry and intracellular development. *Traffic* 5: 561–570.
16. Hashim S, Mukherjee K, Rajc M, Basu S, Mukhopadhyay A (2000) Live *Salmonella* modulate expression of Rab proteins to persist in a specialized compartment and escape transport to lysosomes. *J Biol Chem* 275: 16281–16288.
17. Oh Y, Straubinger R (1996) Intracellular fate of *Mycobacterium avium*: use of dual-label spectrofluorometry to investigate the influence of bacterial viability and opsonization on phagosomal pH and phagosome-lysosome interaction. *Infect Immun* 64: 319–325.
18. Morozova I, Qu X, Shi S, Asamani G, Greenberg J, et al. (2004) Comparative sequence analysis of the *icm/dot* genes in *Legionella*. *Plasmid* 51: 127–147.
19. Delevoeye C, Nilges M, Dautry-Varsat A, Subtil A (2004) Conservation of the biochemical properties of IncA from *Chlamydia trachomatis* and *C. caviae*: oligomerization of IncA mediates interaction between facing membranes. *J Biol Chem* 279: 46896–46906.
20. Barocchi MA, Masignani V, Rappuoli R (2005) Cell entry machines: a common theme in nature? *Nat Rev Microbiol* 4: 349–358.
21. Delevoeye C, Nilges M, Dehoux P, Paumet F, Perrinet S, et al. (2008) SNARE protein mimicry by an intracellular bacterium. *PLOS Pathogens* 4: e1000022.
22. Hackstadt T, Scidmore-Carlson MA, Shaw EI, Fischer ER (1999) The *Chlamydia trachomatis* IncA protein is required for homotypic vesicle fusion. *Cellular Microbiology* 1: 119–130.
23. Fields KA, Fisher E, Hackstadt T (2002) Inhibition of fusion of *Chlamydia trachomatis* inclusions at 32°C correlates with restricted export of IncA. *Infection and Immunity* 70: 3816–3823.
24. Roy CR, Berger KH, Isberg RR (1998) *Legionella pneumophila* DotA protein is required for early phagosome trafficking decisions that occur within minutes of bacterial uptake. *Mol Microbiol* 28: 663–674.
25. Hackstadt T (2000) Redirection of host vesicle trafficking pathways by intracellular parasites. *Traffic* 1: 93–99.
26. Mullock BM, Smith CW, Ihrke G, Bright NA, Lindsay M, et al. (2000) Syntaxin 7 is localized to late endosome compartments, associates with vamp 8, and is required for late endosome-lysosome fusion [In Process Citation]. *Mol Biol Cell* 11: 3137–3153.
27. Nakamura N, Yamamoto A, Wada Y, Futai M (2000) Syntaxin 7 mediates endocytic trafficking to late endosomes. *J Biol Chem* 275: 6523–6529.
28. Scidmore MA, Fischer ER, Hackstadt T (2003) Restricted Fusion of *Chlamydia trachomatis* vesicles with endocytic compartments during the initial stages of infection. *Infection and Immunity* 71: 973–984.
29. Eissenberg LG, Wyrick PB, Davis CH, Rump JW (1983) *Chlamydia psittaci* elementary body envelopes: ingestion and inhibition of phagolysosome fusion. *Infect Immun* 40: 741–751.
30. Belland RJ, Zhong G, Crane DD, Hogan D, Sturdevant D, et al. (2003) Genomic transcriptional profiling of the developmental cycle of *Chlamydia trachomatis*. *PNAS* 100: 8478–8483.
31. de Felipe K, Glove rR, Charpentier X, Anderson O, Reyes M, et al. (2008) *Legionella* eukaryotic-like type IV substrates interfere with organelle trafficking. *PLoS Pathog* 4: e1000117.
32. Rockey DD, Grosenbach D, Hruba D, Peacock M, Heinzen R, et al. (1997) *Chlamydia psittaci* IncA is phosphorylated by the host cell and is exposed on the cytoplasmic face of the developing inclusion. *Mol Microbiol* 24: 217–228.
33. Rockey DD, Viratyosin W, Bannantine JP, Suchland RJ, Stamm WE (2002) Diversity within inc genes of clinical *Chlamydia trachomatis* variant isolates that occupy non-fusogenic inclusions. *Microbiology* 148: 2497–2505.
34. Lutz-Wohlgroth L, Becker A, Brugnara E, Huat Z, Zimmermann D, et al. (2006) *Chlamydiae* in guinea-pigs and their zoonotic potential. *J Vet Med A Physiol Pathol Clin Med* 53: 185–193.
35. Sagi-Eisenberg R (2007) The mast cell: where endocytosis and regulated exocytosis meet. *Immunol Rev* 217: 292–303.
36. Puri N, Roche P (2008) Mast cells possess distinct secretory granule subsets whose exocytosis is regulated by different SNARE isoforms. *PNAS* 105: 2580–2585.
37. Paumet F, Le Mao J, Martin S, Galli T, David B, et al. (2000) Soluble NSF attachment protein receptors (SNAREs) in RBL-2H3 mast cells: functional role of syntaxin 4 in exocytosis and identification of a vesicle-associated membrane protein 8-containing secretory compartment. *J Immunol* 164: 5850–5857.
38. Wu M, Baumgart T, Hammond S, Holowka D, Baird B (2007) Differential targeting of secretory lysosomes and recycling endosomes in mast cells revealed by patterned antigen arrays. *J Cell Sci* 120: 3147–3154.
39. Pryor PR, Mullock BM, Bright NA, Lindsay M, Gray SR, et al. (2004) Combinatorial SNARE complexes with VAMP7 or VAMP8 define different late endocytic fusion events. *EMBO Reports* 5: 590–595.
40. Geisler W, Suchland R, Rockey D, Stamm W (2001) Epidemiology and clinical manifestations of unique *Chlamydia trachomatis* isolates that occupy nonfusogenic inclusions. *J Inf Diseases* 184: 879–884.
41. Xia M, Suchland R, Bumgarner R, Peng T, Rockey D, et al. (2005) *Chlamydia trachomatis* variant with nonfusing inclusions: Growth dynamic and host-cell transcriptional response. *J Inf Diseases* 192: 1229–1236.
42. McNew JA, Parlati F, Fukuda R, Johnston RJ, Paz K, et al. (2000) Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. *Nature* 407: 153–159.
43. Parlati F, Varlamov O, Paz K, McNew JA, Hurtado D, et al. (2002) Distinct SNARE complexes mediating membrane fusion in Golgi transport based on combinatorial specificity. *Proc Natl Acad Sci U S A* 99: 5424–5429.
44. Paumet F, Brugger B, Parlati F, McNew JA, Sollner TH, et al. (2001) A t-SNARE of the endocytic pathway must be activated for fusion. *J Cell Biol* 155: 961–968.
45. Paumet F, Rahimian V, Di Liberto M, Rothman JE (2005) Concerted auto-regulation in yeast endosomal t-SNAREs. *J Biol Chem* 280: 21137–21143.
46. Varlamov O, Volchuk A, Rahimian V, Doege CA, Paumet F, et al. (2004) i-SNAREs: inhibitory SNAREs that fine-tune the specificity of membrane fusion. *J Cell Biol* 164: 79–88.
47. Stebbins C, Galán J (2001) Structural mimicry in bacterial virulence. *Nature* 412: 701–705.
48. Paumet F, Rahimian V, Rothman JE (2004) The specificity of SNARE-dependent fusion is encoded in the SNARE motif. *Proc Natl Acad Sci U S A* 101: 3376–3380.
49. Weber T, Parlati F, McNew JA, Johnston RJ, Westermann B, et al. (2000) SNAREpins are functionally resistant to disruption by NSF and alphaSNAP. *J Cell Biol* 149: 1063–1072.
50. Schwartz L, Austen K, Wasserman S (1979) Immunologic release of β -hexosaminidase and β -glucuronidase from purified rat serosal mast cells. *J Immunol* 123: 1445–1450.
51. Roa M, Paumet F, Le Mao J, David B, Blank U (1997) Involvement of the ras-like GTPase rab3d in RBL-2H3 mast cell exocytosis following stimulation via high affinity IgE receptors (Fc epsilonRI). *J Immunol* 159: 2815–2823.