Current Biology, Vol. 15, 1136–1142, June 21, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.05.046

Structurally Conserved Interaction of Lgl Family with SNAREs Is Critical to Their Cellular Function

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Summary

The Lethal giant larvae (Lgl) tumor suppressor family is conserved from yeast to mammals and plays a critical yet controversial role in cell polarity. Studies on *Drosophila* Lgl suggest that its function in polarity is through regulation of the acto-myosin cytoskeleton. In contrast, studies on the yeast Lgl homologs, Sro7/Sro77, suggest a function in exocytosis through interaction with the t-SNARE Sec9. Using yeast/mammalian Lgl chimeras, we demonstrate that the overall architecture of Lgl proteins is highly conserved and that the C-terminal domain is the major site of SNARE interaction within both yeast and mammalian homologs. Importantly, we find that the ability of Lgl chimeras to function as the only source of Lgl in yeast correlates precisely with the ability to interact with the yeast t-SNARE. We report a novel interaction between Sro7 and the yeast myosin V, Myo2. However, we find that interactions with either Myo2 or Myo1 (myosin II) cannot account for the dramatic functional differences observed for these chimeras in yeast. These results provide the first demonstration that the interaction of an Lgl family member with a specific effector is critical to its function in vivo. These data support the model that the Lgl family functions in cell polarity, at least in part, by regulating SNARE-mediated membrane delivery events at the cell surface.

Results and Discussion

Chimeric Lgl Proteins Implicate the C-terminal Domain in Determining the Specificity of SNARE Binding

To determine the region within Sro7 involved in binding to the t-SNARE Sec9, we compared full-length Sro7 to the Sro7 C-terminal domain in their ability to bind to recombinant full-length Sec9. The results shown in Figure 1A demonstrate that whereas 14% of the in vitro translated Sro7-CT protein bound to GST-Sec9, 90% of the full-length Sro7 was bound to Sec9. Binding assays with the Sro7 N-terminal WD domain showed no detectable binding to Sec9 (A.G. and P.B., unpublished data). Previous work from our lab has shown that whereas the C-terminal half of Sro7 was capable of binding in vitro to Sec9, only full-length Sro7 was capable of interacting with Sec9 in an in vivo assay [1]. This suggests that although the C-terminal domain of Sro7 is competent for binding to the t-SNARE Sec9, its ability to bind in vitro (and in vivo) is dramatically enhanced by the presence of the N-terminal WD40 repeat domain.

Our finding that mammalian Mlgl-1 interacts with the mammalian t-SNARE Syntaxin 4 suggests that SNARE interaction is a conserved property of the members of this family [2]. In order to examine the SNARE binding capacity and specificity of Sro7/Lgl family proteins, we investigated whether Sro7 and Mlgl-1 interact with the heterologous t-SNAREs (i.e., Sro7 with Syntaxin 4 and Mlgl-1 with Sec9). Not surprisingly, given the evolutionary distance between yeast and mammals, the noncognate pairs of proteins did not interact (Figure 1D). We made use of this specificity to extend the domain analysis by constructing chimeric molecules, in which the N-terminal WD repeats of the yeast and mammalian proteins were reciprocally exchanged (Figure 1B). This allowed us to examine the SNARE binding activity of each domain in the context of the full-length protein. This swap produced two chimeric proteins: Sro7$^{1–525}$/Mlgl$^{463–1034}$ (referred to as the S/M chimera) and Mlgl$^{1–462}$/Sro7$^{526–1033}$ (M/S chimera) (Figure 1B). Each chimeric protein was in vitro translated and bound to a panel of recombinant SNARE proteins to assess its binding properties (Figures 1C and 1D). Remarkably, we find that the binding of the M/S chimera to Sec9 was nearly identical to that of wild-type full-length Sro7 (Figure 1D). Like Sro7, the M/S chimera showed the strongest interaction with full-length Sec9. A slight interaction of the M/S chimera with Syntaxin 4 was also observed. Conversely, the S/M chimera demonstrated a clear preference for Syntaxin 4, although a weak interaction with Sec9 was also detected (Figure 1D). These findings demonstrate that the specificity of the Lgl-SNARE interaction resides largely in the C-terminal domain of the Lgl family. Although the N terminus of Sro7/Lgl appears to have a structural role in potentiating the overall affinity of the interaction with t-SNAREs, it has only a minor role in determining its specificity. These data provide strong evidence that the C-terminal domain of the Lgl family has a critical role in mediating the specific interaction with SNARE proteins in both yeast and mammalian cells.

We next determined the ability of each of the chimeras to function in vivo as the only source of Sro7/Lgl. Wild-type and chimeric sequences were introduced into yeast on both single-copy (*CEN*) and multicopy (2μ) vectors under the control of the *SRO7* promoter, and their expression levels were monitored by quantitative immunoblot analysis with antisera specific to the N and C termini of Sro7 or the C terminus of Mlgl-1. As expected, the expression level of *SRO7* from a *CEN* plasmid was identical to that from the chromosomal locus (data not shown); each of the chimeric proteins was also expressed, although at a lower level (Figure 2A). When the chimeras were introduced on multicopy (2μ) vectors, their expression improved considerably: Quantitative immunoblot experiments revealed that each of

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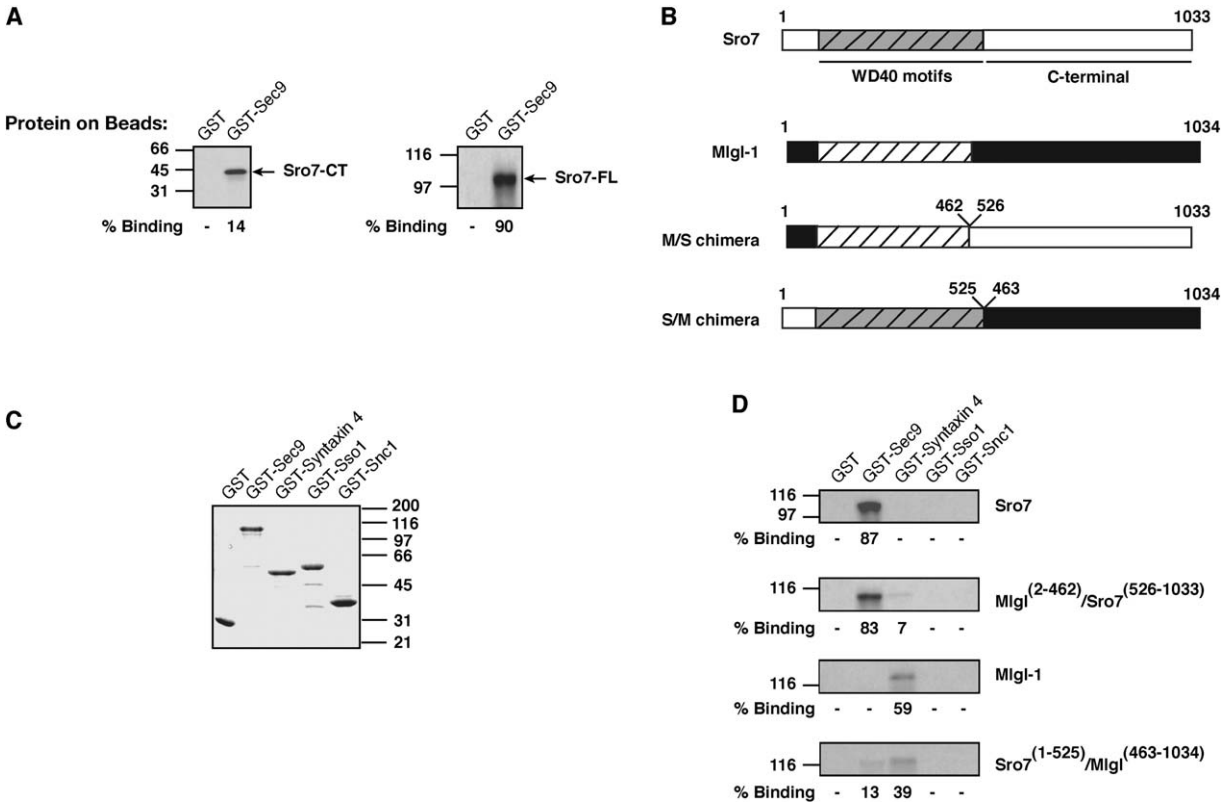

Figure 1. Lgl Family of Proteins Interacts with Their Cognate Plasma Membrane SNARE via the C-Terminal Domain In Vitro

(A) Binding of Sro7-CT and Sro7-FL proteins to GST-Sec9. Sro7-CT and Sro7-FL were in vitro transcribed and translated with rabbit reticulate lysate in the presence of [^{35}S]methionine. The radiolabeled proteins were incubated with 2 μM GST or GST-Sec9 immobilized on beads. After several washes, bound and unbound samples were analyzed by SDS-PAGE and autoradiography and quantitated with Storm PhosphorImager. The percentages of in vitro translated proteins bound to GST-Sec9 are indicated.

(B) Schematic representations of domain structure of Sro7 and Mlgl-1 and chimeras used in this study. The conserved WD repeat domain in Sro7 and Mlgl-1 is shown as hatched.

(C) Coomassie staining of the GST-SNARE recombinant proteins used in the in vitro binding assays.

(D) Binding of Sro7, Mlgl-1, or chimeric proteins to GST-SNARE fusion proteins. Sro7, Mlgl-1, and the chimeras were in vitro transcribed and translated with rabbit reticulate lysate in the presence of [^{35}S]methionine and incubated with various GST fusions proteins (2 μM). Bindings were performed as described in the Experimental Procedures. The percentages of in vitro translated proteins bound to GST-SNAREs are indicated.

the chimeras was expressed at levels that were roughly similar (2-fold higher) to that of single-copy Sro7.

The critical test of function for the Sro7/Lgl chimeras is their ability to rescue the severe cold-sensitive growth and secretion defects that are manifest in the *sro7Δ,sro77Δ* mutant. Figure 2B demonstrates that when expressed from a high-copy 2μ plasmid, the M/S chimera is able to suppress the cold-sensitive phenotype of this mutant, nearly as strongly as wild-type Sro7 (Figure 2B). When present on a *CEN* plasmid, the M/S chimera is a weak suppressor of the *sro7Δ,sro77Δ* mutant (Figure 2B). In contrast, the S/M chimera was unable to suppress the cold sensitivity of this strain, even when expressed at levels similar to those of the M/S chimera (Figure 2B). We also examined the ability of these chimeras to suppress the post-Golgi secretory defect associated with loss of chromosomal *SRO7* and *SRO77* genes by taking advantage of the Bgl2 secretion assay [3]. Bgl2 is a cell-wall endoglucanase whose delivery to the cell surface depends on the intact secretory pathway [4]. Defect in the normal transport of Bgl2 manifests itself as an increase in the internal pool of this protein in relation to the extracellular pool. We found that strains containing empty vector or 2μ plasmids expressing Mlgl-1 and S/M proteins all had a pronounced defect in the secretion of Bgl2 protein (Figure 2C). In each case, the secretion defect was markedly exacerbated by a shift to low temperature. In contrast, in strains expressing Sro7 from a *CEN* plasmid and the M/S chimera from a 2μ plasmid (which results in similar levels of each protein), the secretory function was restored both at permissive and restrictive conditions (Figure 2C). Therefore, the presence of the Sec9 binding domain at the C terminus appears to be critical for its ability to restore both growth and secretory function within the cell.

To determine whether the gain of secretory function by the chimeras involved a restoration of the interaction with Sec9, we performed coimmunoprecipitation assays on strains containing each of the chimeras. Sro7,

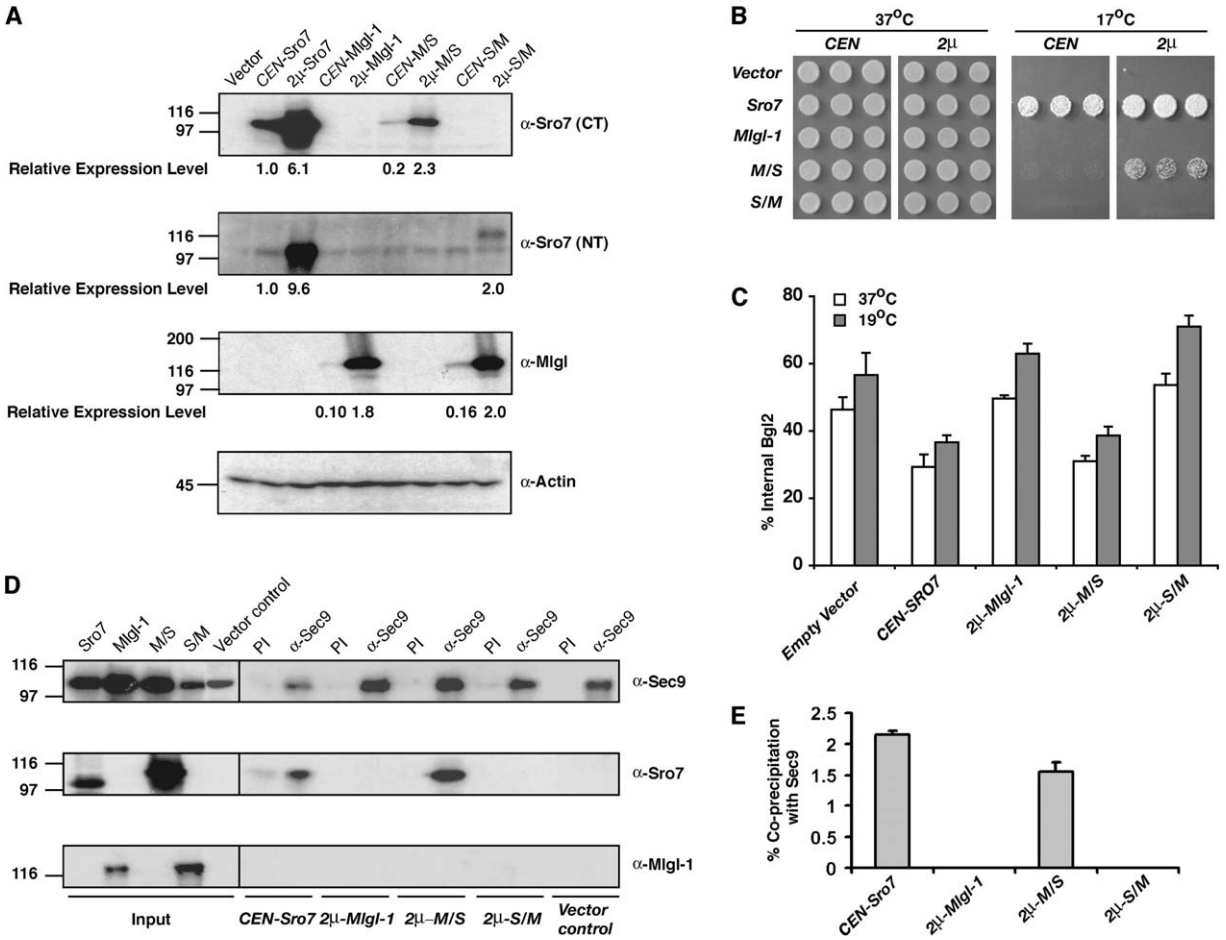

Figure 2. Rescue of the Cold-Sensitive Phenotype and the Post-Golgi Secretory Defect of the *sro7Δ,sro77Δ* Double Mutant by the Mlgl/Sro7 Chimera

(A) Expression of chimeric proteins in *S. cerevisiae*. Yeast extracts from an *sro7Δ,sro77Δ* strain expressing the indicated genes from a low- (*CEN*) or high-copy (2μ) plasmid were immunoblotted with antibodies against the C and N terminus of Sro7, Mlgl-1, or actin. The expression levels of proteins in relation to *CEN-SRO7* are indicated.

(B) The Mlgl/Sro7 chimera can rescue the cold sensitivity of an *sro7Δ,sro77Δ* strain. Chimeras were expressed in an *sro7Δ,sro77Δ* strain from a low- or high-copy plasmid, and the different strains were tested for growth at permissive (37°C) and restrictive temperature (17°C) on YPD media (1% Bacto yeast extract, 2% Bacto peptone, and 2% glucose).

(C) Analysis of Bgl2 protein distribution in the *sro7Δ,sro77Δ* mutant expressing *M/S* or *S/M* chimera. The percentage of the total Bgl2 that is found internally in different strains grown at 37°C or shifted to 19°C for 3 hr is depicted graphically. The data represent the average of three independent experiments.

(D) Mlgl/Sro7 chimera interacts specifically with the t-SNARE Sec9 in vivo. *SEC9* was expressed from a multicopy plasmid in an *sro7Δ,sro77Δ* strain containing either *SRO7* on a low-copy vector or *Mlgl-1*, *M/S*, and *S/M* on high-copy vectors. Lysates from each strain were subjected to immunoprecipitation with either saturating amounts of affinity-purified antibodies to Sec9 or an equivalent amount of IgG purified from preimmune (PI) sera of the same rabbit. The immune complexes were captured, washed, and analyzed by blotting with α-Sro7, α-Mlgl-1, and α-Sec9 antibodies.

(E) Quantitation of the coimmunoprecipitation efficiencies with a Storm PhosphorImager with ImageQuant software. The values are expressed as the amount of coimmunoprecipitating proteins present as a percentage of the total protein in the lysate.

Mlgl-1, and the two chimeric proteins were expressed in the *sro7Δ,sro77Δ* genetic background, so that the plasmid-encoded proteins were the only source of Sro7/77 in the yeast cells. Detergent lysates were prepared from each strain and subjected to immunoprecipitation with purified antiserum raised against Sec9. We monitored the presence of each protein in the immunoprecipitation by Western blotting with antibodies against Sec9, Sro7 (C terminus), or Mlgl-1. The results shown in Figures 2D and 2E demonstrate that Sro7 and the M/S chimera clearly associated with Sec9 in cell lysates, whereas Mlgl-1 and the S/M chimera did not. These data provide the first direct evidence that SNARE binding is likely to be a critical and conserved component of the in vivo function of the Sro7/Lgl family of proteins. Furthermore, we demonstrate that SNARE binding capacity of two highly divergent Lgl family members is dependent on amino acid sequence determinants localized within their C-terminal domain.

Recently, Mlgl-1 has been shown to complement the *D. melanogaster* Lgl mutant defect when expressed as a transgene [5]. In order to determine whether the ability to interact with Syntaxin 4 was also conserved between mouse and fly Lgl isoforms, we examined the

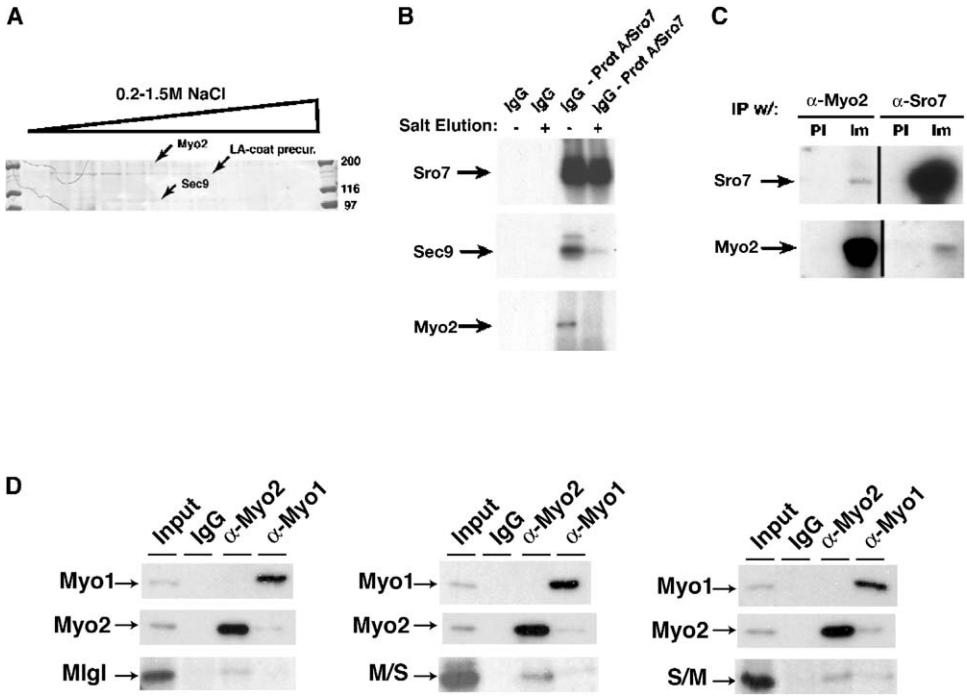

Figure 3. Characterization of Myosin V and Myosin II Interactions with Sro7/Lgl Chimeras

(A) Isolation of Sro7-associated proteins from yeast lysates. Protein-A-tagged Sro7 was isolated by affinity binding to IgG Sepharose from native yeast detergent extracts. Beads were washed, and associated proteins were eluted with a 0.2–1.5 M salt gradient. The marked bands were identified by mass spectrometry analysis of trypsin-digested gel slices.

(B) Immunoblot analysis of proteins specifically associated with ProtA/Sro7 beads and absent from control IgG Sepharose beads incubated with a lysate containing an untagged form of Sro7.

(C) Coimmunoprecipitation of Sro7 and Myo2 from detergent extracts of wild-type yeast containing single-copy Sro7 and Myo2. Identical exposures are shown for each immunoblot.

(D) Coimmunoprecipitation of Mlgl-1 and M/S or S/M chimeric proteins with α-Myo2 and α-Myo1 from detergent extracts of *sro7Δ,sro77Δ* strains expressing Mlgl-1, M/S, or S/M on high-copy vectors. Quantitation of the efficiency of coprecipitations demonstrated approximately 1%–2% of the total Mlgl-1, M/S, or S/M chimera associated with the Myo2 immunoprecipitations.

ability of full-length and C-terminal domains of Mlgl-1 and Lgl proteins to interact with GST-Syntaxin 4 in vitro. As can be seen in Figure S1 (see the Supplemental Data available with this article online), we find that *Drosophila* Lgl has retained the ability to specifically bind Syntaxin 4, although to a slightly lesser degree than that of the cognate Mlgl-1 protein. Interestingly, the C-terminal domains of both of these proteins interact with Syntaxin 4 at a level similar to that of the full-length protein. Therefore, the ability of the C termini of Lgl family members to bind t-SNAREs is a highly conserved property of this family of proteins.

Interaction of Sro7 and Sro7/Mlgl Chimeras with Type II and Type V Myosins

In order to identify additional binding partners of Sro7 within the yeast cell, we performed a proteomic analysis of the major proteins that coassociated with a Protein A-Sro7 fusion protein. We found three major proteins that were specifically enriched in the high-salt elution of Protein A-Sro7 by MALDI-TOF mass spectrometry analysis of proteolyzed gel slices (Figure 3A). The most abundant was identified as the 150 kd precursor of the LA viral coat protein—a common contaminant in IgG-Protein A purifications from yeast. The next two abundant proteins were unambiguously identified as the t-SNARE Sec9 and the type V myosin, Myo2, which is involved in the polarized delivery of post-Golgi vesicles. The identity and specificity of these proteins were confirmed by immunoblot analysis of IgG beads before and after the salt elution along with IgG beads incubated with a control lysate lacking Protein A-Sro7 (Figure 3B). We also confirmed the coassociation of Myo2 with Sro7 by reciprocal coimmunoprecipitation analysis from wild-type cells expressing only endogenous Sro7 and Myo2 (Figure 3C). This identifies a novel interaction between Sro7 and the yeast type V myosin, Myo2. With use of recombinant proteins, the interaction between Sro7 and Myo2 was found to be direct and mapped to the neck region of Myo2 (G.R., unpublished data). Whereas full-length Sro7 binds avidly to recombinant GST-Myo2 neck protein, the Sro7 C-terminal domain failed to show detectable interaction, suggesting that the N-terminal WD domain is required for this interaction (Figure S2). Kagami et al. [6] have previously suggested a physical interaction between the yeast Type II myosin, Myo1, and Sro7; however, we were unable to detect Myo1 in any of the samples shown in Figures 3A–3C. We were, however, able to detect a weak association between Sro7/Lgl proteins and Myo1 in Myo1 immunoprecipitations (see below).

To determine whether the loss of myosin II or myosin

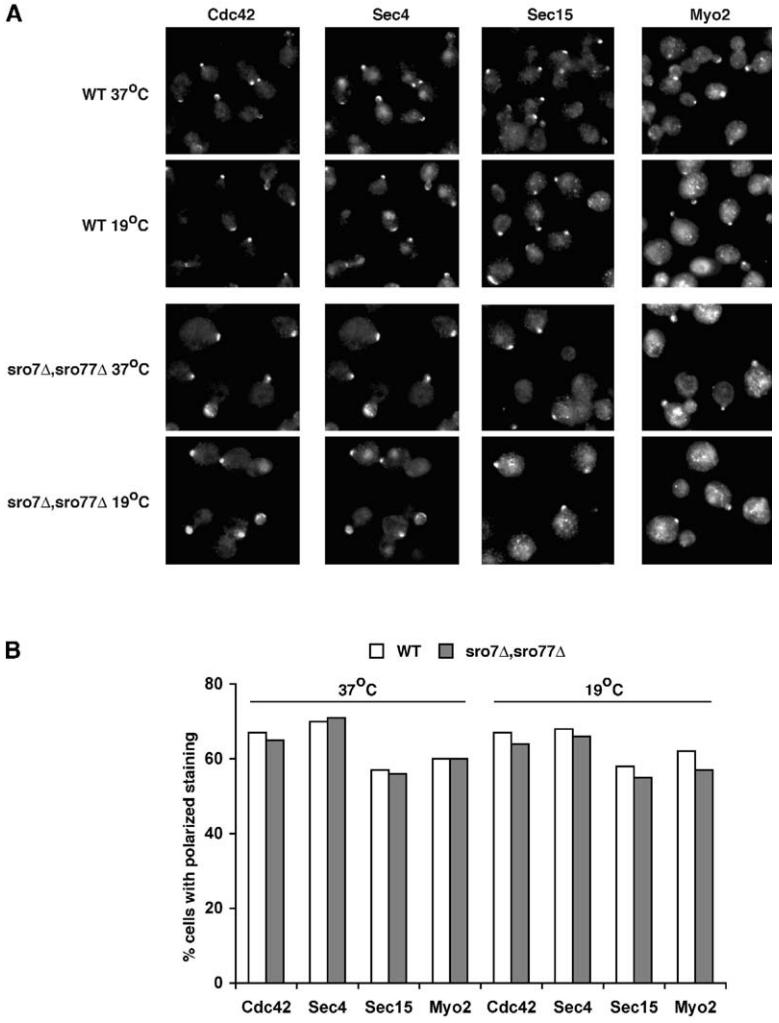

Figure 4. Distribution of Polarity and Secretion Markers Cdc42, Sec4, Sec15, and Myo2 Is Unaffected in *sro7Δ,sro77Δ* Cells at Both Permissive and Restrictive Temperatures

(A) Wild-type (WT) and *sro7Δ,sro77Δ* cells were grown at 37°C or shifted to 19°C for 3 hr before fixation and then processed for fluorescence microscopy. Visualization of the polarity-establishment protein Cdc42, the Rab GTPase Sec4, the exocyst subunit Sec15, and the vesicle-transport motor Myo2 was performed with specific purified antibodies.

(B) Quantitation of polarized markers in WT and *sro7Δ,sro77Δ* cells. Cells were scored for polarized localization of the various markers at the emerging bud sites, bud tips, or mother-daughter neck regions. A minimum of 200 cells were counted for each experiment.

V binding could account for the functional differences we see in the S/M versus M/S chimeras, we examined the ability of each of these proteins and Mlgl-1 to be coprecipitated by antibodies directed against yeast Myo1 or Myo2 proteins. As observed in Figure 3D, Mlgl-1 and both S/M and M/S chimeras could clearly associate with Myo2 and weakly with Myo1 immunoprecipitates. Therefore, the ability to interact with either of these myosins appears to be highly conserved; however, these interactions cannot account for the functional differences seen in these chimeras. The dramatic functional and SNARE-interaction difference seen with the M/S and S/M chimeras provides strong evidence that the ability to interact with the SNARE protein is critical to the in vivo function of Sro7.

The Localization of Actin-Dependent Secretory and Polarity Markers Is Unaffected in *sro7Δ,sro77Δ* Cells

We have previously demonstrated that actin polarity is not detectably altered under conditions in which we see a dramatic defect in secretory function in *sro7Δ, sro77Δ* cells [1]. Recently, it has been suggested that the exocytic defects observed in *sro7Δ,sro77Δ* cells might arise as a secondary effect of actin-polarization defects [7]. To determine whether there was a subtle effect, not previously detected in *sro7Δ,sro77Δ*, on actin polarity, we examined the localization of a number of proteins whose polarity is known to be highly sensitive to perturbations in actin polarity/integrity. These include the markers of polarized growth in yeast: Cdc42, Sec4, Sec15, and Myo2. The localization of these markers is sensitive to both the integrity of actin cables and ongoing secretion [8, 9]. We examined the localization of Cdc42, Sec4, Sec15, and Myo2 in wild-type cells and cells that lack functional copies of Sro7 and Sro77 with affinity-purified antibodies against each protein at both permissive (37°C) and restrictive temperature (19°C). We observed that in *sro7Δ,sro77Δ* mutant cells, the staining pattern for all four proteins examined was similar to wild-type cells before and after a 3 hr shift to the restrictive temperature (Figure 4). This finding is consistent with our previous observation that the actin cytoskeleton organization in this mutant is normal after a 3 hr downshift, although a dramatic effect on growth and secretion is clearly evident under these conditions [1]. Moreover, because Myo2 polarization is normal in *sro7Δ,sro77Δ* cells, this finding demonstrates that the interaction of Myo2 and Sro7 is not required for Myo2

localization. Importantly, it reaffirms that the secretory defect associated with *sro7Δ,sro77Δ* cells is not an indirect result of an actin depolarization. In contrast, we believe that the actin-polarity defects observed by Aronov et al. [7] are likely to be an effect of growing the yeast cells at 25°C—a semirestrictive temperature for this mutant—prior to the low-temperature shift.

Conclusion

Two major models for the function of the Sro7/Lgl family of proteins have been proposed. The first model suggests that Sro7/Lgl proteins work to regulate cell-surface polarity through modulation of acto-myosin function [6, 10, 11]. Critical to this model is the observation of a conserved interaction of Lgl family proteins with type II myosins. We have suggested a second, distinct but not mutually exclusive model, in which the action of Sro7/Lgl proteins is directed at regulation of vesicle transport to the cell surface. Each of these models is based, in part, on the physical association of Sro7/Lgl protein with a specific candidate effector. In this paper, we have generated the first direct test of the importance of the interaction of an Lgl family protein with a specific effector. Here, we confirm that Sro7 interacts with the t-SNARE Sec9 and also describe a novel and conserved interaction with a yeast Type V myosin, Myo2. Most importantly, we find that the ability of Sro7 to bind to the yeast t-SNARE Sec9 appears to be critical to the ability of Lgl chimeric proteins to functionally rescue the growth and secretion defects associated with loss of Sro7/77. The observed interaction of Myo2 with Sro7 was also observed between Myo2 and Mlgl-1, as well as both the Sro7/Mlgl chimeras. This interaction, therefore, cannot account for the functional differences seen between the chimeras. Consistent with the notion that Lgl proteins act in vesicle transport, we find that the interaction of the C-terminal domain of Lgl family members with SNAREs appears to be a highly conserved property of all the members of this family, including the *Drosophila* tumor suppressor Lgl—although the native SNARE partner in flies has not yet been identified. Taken together, these findings provide strong evidence that the ability to bind t-SNAREs is a crucial component of the functional architecture of Lgl proteins. In yeast, it is clear that loss of this protein leads to dramatic but not complete loss of secretory function. Likewise in mammals and flies, Lgl function may be restricted to the delivery of a distinct class of membrane vesicles, perhaps containing other key cell-fate determinants. Furthermore, the interaction with type V myosins (and perhaps type II myosins) may play a role either in movement of these specific vesicles or perhaps in the polarization of the Lgl protein itself on the plasma membrane. Future work will no doubt help to elucidate the details of how these conserved interactions are mechanistically tied to the function of Lgl proteins in cell polarity.

Experimental Procedures

Purification of the Recombinant Proteins

GST fusion proteins were prepared as described previously [12]. See the Supplemental Experimental Procedures for the protocol used for purification of full-length Sec9.

In Vitro Transcription-Translation

Amplified DNA was added directly to reticulocyte lysate-coupled in vitro transcription-translation system (TNT T7 Quick for PCR DNA, Promega) in the presence of [^{35}S]methionine and processed as described by the manufacturer.

Binding Assays

Binding assays were done as described previously [1]. See the Supplemental Experimental Procedures for details.

Identification of Sro7-Associated Proteins

Identification of the major proteins associated with Protein-A-tagged Sro7 was as described in the Supplemental Experimental Procedures.

Native Immunoprecipitations

Native coimmunoprecipitations were performed as described previously [1] with affinity-purified antibodies to Sec9 and Sro7. Detailed protocol for immunoprecipitations of chimeras with α-Myo1 and α-Myo2 is described in the Supplemental Experimental Procedures.

Bgl2-Secretion Assay

Exoglucanase Bgl2 secretion was tested essentially as described previously [3].

Construction of Chimeras

Chimeric molecules were constructed by overlapping PCR and verified by sequencing [13, 14]. Mgl-1 cDNA was amplified from reverse-transcribed total mouse Kidney RNA, and sequence analysis demonstrated a perfect match to the published sequence (Genbank accession number NM_008502). Primers and templates used are described in Table S1.

Supplemental Data

Detailed Experimental Procedures, as well as several supplemental figures and tables, are available at http://www.current-biology.com/cgi/content/full/15/12/1136/DC1/.

Acknowledgments

We thank the members of the Brennwald laboratory for helpful discussions and critical reading of the manuscript. The authors wish to thank Dr. James McNew for helpful suggestions in the purification of full-length Sec9 and the University of North Carolina Proteomics Facility for mass spectrometry analysis. This work was supported by National Institutes of Health grant R01GM-54712 (to P.B.).

Received: December 15, 2004
Revised: May 9, 2005
Accepted: May 10, 2005
Published: June 21, 2005

References

1. Lehman, K., Rossi, G., Adamo, J.E., and Brennwald, P. (1999). Yeast homologues of tomosyn and lethal giant larvae function in exocytosis and are associated with the plasma membrane SNARE, Sec9. J. Cell Biol. *146*, 125–140.
2. Musch, A., Cohen, D., Yeaman, C., Nelson, W.J., Rodriguez-Boulan, E., and Brennwald, P.J. (2002). Mammalian homolog of Drosophila tumor suppressor lethal (2) giant larvae interacts with basolateral exocytic machinery in Madin-Darby canine kidney cells. Mol. Biol. Cell *13*, 158–168.
3. Adamo, J.E., Rossi, G., and Brennwald, P. (1999). The Rho GTPase Rho3 has a direct role in exocytosis that is distinct from its role in actin polarity. Mol. Biol. Cell *10*, 4121–4133.
4. Harsay, E., and Bretscher, A. (1995). Parallel secretory pathways to the cell surface in yeast. J. Cell Biol. *131*, 297–310.
5. Grifoni, D., Garoia, F., Schimanski, C.C., Schmitz, G., Laurenti, E., Galle, P.R., Pession, A., Cavicchi, S., and Strand, D. (2004). The human protein Hugl-1 substitutes for Drosophila lethal gi-

ant larvae tumour suppressor function in vivo. Oncogene *23*, 8688–8694.

6. Kagami, M., Toh, E.A., and Matsui, Y. (1998). Sro7p, a *Saccharomyces cerevisiae* counterpart of the tumor suppressor l(2)gl protein, is related to myosins in function. Genetics *149*, 1717–1727.
7. Aronov, S., and Gerst, J.E. (2004). Involvement of the late secretory pathway in actin regulation and mRNA transport in yeast. J. Biol. Chem. *279*, 36962–36971.
8. Pruyne, D.W., Schott, D.H., and Bretscher, A. (1998). Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. J. Cell Biol. *143*, 1931–1945.
9. Irazoqui, J.E., Howell, A.S., Theesfeld, C.L., and Lew, D.J. (2005). Opposing roles for actin in Cdc42p polarization. Mol. Biol. Cell *16*, 1296–1304.
10. Strand, D., Raska, I., and Mechler, B.M. (1994a). The Drosophila lethal(2)giant larvae tumor suppressor protein is a component of the cytoskeleton. J. Cell Biol. *127*, 1345–1360.
11. Strand, D., Jakobs, R., Merdes, G., Neumann, B., Kalmes, A., Heid, H.W., Husmann, I., and Mechler, B.M. (1994b). The Drosophila lethal(2)giant larvae tumor suppressor protein forms homo-oligomers and is associated with nonmuscle myosin II heavy chain. J. Cell Biol. *127*, 1361–1373.
12. Rossi, G., Salminen, A., Rice, L.M., Brunger, A.T., and Brennwald, P. (1997). Analysis of a yeast SNARE complex reveals remarkable similarity to the neuronal SNARE complex and a novel function for the C terminus of the SNAP-25 homolog, Sec9. J. Biol. Chem. *272*, 16610–16617.
13. Horton, R.M., Hunt, H.D., Ho, S.N., Pullen, J.K., and Pease, L.R. (1989). Engineering hybrid genes without the use of restriction enzymes: Gene splicing by overlap extension. Gene *77*, 61–68.
14. Yon, J., and Fried, M. (1989). Precise gene fusion by PCR. Nucleic Acids Res. *17*, 4895.