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The Yeast Kinesin-related Protein Smy1p Exerts Its Effects on the Class V Myosin Myo2p via a Physical Interaction

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We have discovered evidence for a physical interaction between a class V myosin, Myo2p, and a kinesin-related protein, Smy1p, in budding yeast. These proteins had previously been linked by genetic and colocalization studies, but we had been unable to determine the nature of their association. We now show by two-hybrid analysis that a 69-amino acid region of the Smy1p tail interacts with the globular portion of the Myo2p tail. Deletion of this myosin-binding region of Smy1p eliminates its ability to colocalize with Myo2p and to overcome the *myo2–66* mutant defects, suggesting that the interaction is necessary for these functions. Further insights about the Smy1p–Myo2p interaction have come from studies of a new mutant allele, *myo2–2,* which causes a loss of Myo2p localization. We report that Smy1p localization is also lost in the *myo2–2* mutant, demonstrating that Smy1p localization is dependent on Myo2p. We also found that overexpression of Smy1p partially restores myo2–2p localization in a myosin-binding region–dependent manner. Thus, overexpression of Smy1p can overcome defects in both the head and tail domains of Myo2p (caused by the *myo2–66* and *myo2–2* alleles, respectively). We propose that Smy1p enhances some aspect of Myo2p function, perhaps delivery or docking of vesicles at the bud tip.

INTRODUCTION

In *Saccharomyces cerevisiae*, virtually all growth is directed to the bud rather than the mother cell. Myo2p is a class V myosin that has been implicated in this polarized growth. The temperature-sensitive *myo2–66* mutant fails to target growth to the bud at restrictive temperature, resulting in abnormally large mother cells (Johnston *et al.,* 1991). Because vesicles were observed to accumulate in the mutant, Johnston *et al.* (1991) proposed that Myo2p targeted growth by delivering secretory vesicles to the bud. However, evidence that Myo2p is indeed a secretory vesicle motor is far from conclusive (Liu and Bretscher, 1992; Govindan *et al.*, 1995). Immunolocalization studies also implicate Myo2p in polarized growth. Myo2p normally localizes to sites of active growth, such as the bud tip and the mother–daughter neck during cytokinesis (Lillie and Brown, 1994). Myo2p also has been implicated in the delivery of vacuoles to the emerging bud (Hill *et al.*, 1996; Catlett and Weisman, 1998).

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Abbreviations used: IgG, immunoglobulin G; MBR, myosin-binding region; MCS, multiple cloning site.

Much to our surprise, a search for suppressors of the *myo2–66* defect led to the discovery of Smy1p, a rather divergent member of the kinesin superfamily (Lillie and Brown, 1992). It was not immediately clear how overexpression of a putative microtubule-based motor protein would compensate for a defect in an actin-based motor protein (Myo2p). Further investigation showed that Smy1p itself is not required for polarized growth, because deletion of *SMY1* causes no detectable phenotypic change. Nor can Smy1p completely replace Myo2p function, inasmuch as *MYO2* is essential. We have ruled out the possibility that suppression is an artifact of overexpression; if *SMY1* is deleted and the only form of Myo2p present is encoded by *myo2–66*, the cell is dead even at permissive temperatures (synthetic lethality). Furthermore, we have eliminated the obvious possibility that Smy1p provides an alternate pathway along microtubules (Lillie and Brown, 1998). Myo2p and Smy1p colocalize and their localizations are perturbed in an identical way by several cellular stresses (Lillie and Brown, 1994). Overexpression of Smy1p not only restores myo2–66p localization, but also enhances the localization of wild-type Myo2p. However, Myo2p can localize independently of Smy1p, because deletion of Smy1p does not abolish the localization of Myo2p.

Therefore, it seems clear that Smy1p is in close proximity to Myo2p and acts rather directly to enhance Myo2p func-

Table 1. Yeast strains used in this study

Strain	Relevant genotype[a]	Source
Y190	*MAT***a** *gal4 gal80 leu2-3,112 trp1-901 his3 ade2-101 URA*::*GAL1*[b]*-lacZ LYS2*::*GAL1-HIS3*	S. Elledge
L40	*MAT***a** *leu2 trp1 his3 LYS2*::*lexA-HIS3 URA3*::*lexA*[c]*-lacZ*	R. Sternglanz
SLY88	*MAT***a** *myo2-66 ura3-52 leu2-3,112 trp1-Δ1 his3 his6*	
	MATα myo2-66 ura3-52 leu2-3,112 TRP1 HIS3 HIS6	Lillie and Brown (1994)
SLY86	*MAT***a** *smy1-Δ2*::*LEU2 ura3-52 leu2-3,112 trp1-289 his4*	
	MATα smy1-Δ2::*LEU2 ura3-52 leu2-3,112 TRP1 his4*	Lillie and Brown (1994)
LWY2599	*MAT***a** *myo2-2 his3Δ-200 lys2-801 leu2 trp1-Δ901 suc2-Δ9 ade8*::*HIS3*	L. Weisman
LWY7213	*MAT***a** *MYO2 his3Δ-200 lys2-801 leu2 trp1-Δ901 suc2-Δ9 ade8*::*HIS3*	L. Weisman

[a] Some strains may carry additional mutations; most or all are S288C derivatives and therefore *gal2*.
[b] Contains 4 Gal4 DNA-binding sites within the GAL1 promoter (Clontech, Palo Alto, CA).
[c] Contains 8 LexA DNA-binding sites within the lexA promoter (Dagher and Filhol-Cochet, 1997).

tion independent of microtubules; however, the mechanism of action remained a mystery. We now have gained the first insight into how Smy1p exerts its effects on Myo2p. In this article we report that these proteins interact in the two-hybrid system. We have mapped the site of interaction and present evidence that the interaction site is necessary for Smy1p localization and for the suppression of *myo2–66* by *SMY1*. Our data indicate that the physical interaction of a kinesin-related protein can cause physiological changes in the behavior of a myosin.

MATERIALS AND METHODS

Yeast Strains and Media

All yeast strains used are listed in Table 1. Liquid media used were rich medium YPD and synthetic complete medium SC lacking the appropriate supplement to select for plasmid (Sherman *et al.,* 1986). Standard (Sherman *et al.,* 1986) solid media were made by adding 1.5% agar to YPD or synthetic complete medium. For all media, glucose was autoclaved separately and added to 2%. Cultures were grown at 30°C unless otherwise indicated.

DNA Manipulations

Standard procedures were used for DNA manipulations and *Escherichia coli* transformation (Sambrook *et al.,* 1989) and for yeast transformation by the lithium acetate method (Sherman *et al.,* 1986).

Plasmids YEpSMY1–26 and YEpSMY1–38 contain the full-length *SMY1* gene in the high-copy-number vectors YEp352 (2μ *URA3*) and YEp351 (2μ *LEU2*), respectively (Lillie and Brown, 1994). To create YEpSMY1$_{578}$, YEpSMY1–26 was digested with *Sst*I and religated, (9 bp of the vector are read before a stop codon is reached). An *LEU2* version of this construct was made by inserting an *Sst*I fragment from YEpSMY1$_{578}$ into the *Sst*I site of YEp351 (Hill *et al.*, 1986), creating the plasmid YEpSMY1$_{578}$-351 used in Figure 6. YEpSMY1$_{647}$ was created by inserting a *Hpa*I-*Sca*I fragment of YEpSMY1–26 into the *Sma*I site of YEp352 (99 bp are read before a stop codon is encountered). Protein expression levels of Smy1p were comparable from all plasmids, as detected by Western analysis and displayed in Figure 4B.

Two-Hybrid Vectors and Constructs

The Gal4 two-hybrid activation domain vectors pGAD-C(x), where x = 1, 2, or 3 to indicate reading frame, were kindly provided by Philip James (University of Wisconsin, Madison, WI) (James *et al.*, 1996). Each of these vectors has a multiple cloning site (MCS) in a different reading frame, a high copy number (2μ *LEU2*), and an altered *ADH* promoter yielding lower expression levels than pACTII (another activation domain vector; see below). The lower expression level is useful for avoiding toxicity effects.

The vector pBTM116 (2μ *TRP1*) is a high-copy-number, two-hybrid LexA DNA-binding domain vector (Bartel *et al.*, 1993). For these studies we have created three new versions, each containing the MCS in a different reading frame. This series referred to as pBTM-C(x), where x = 1, 2, or 3 was made by inserting the appropriate *Eco*RI–*Pst*I MCS fragment from the pGAD-C(x) series into the *Eco*RI–*Pst*I sites of pBTM116.

pAS1-CYH2 (2μ *TRP1*) and pACTII (2μ *LEU2*) are two-hybrid Gal4 DNA binding domain and activation domain vectors, respectively. Both are high-copy-number plasmids containing strong *ADH* promoters (Clontech, Palo Alto, CA).

All two-hybrid constructs used in this study are listed in Table 2. The plasmids listed as fragment sources are as follows: plasmid YEpSMY1–26 was described above. PKS$^-$D9 contains the *SMY1* gene (*Sal*I–*Pst*I) in Bluescript (Stratagene, La Jolla, CA). pNLC10 was obtained from N. Catlett and L. Weisman. It contains an ~1.6-kbp *Spe*I–*Cla*I tail fragment of *myo2–2* in Bluescript.

Two-Hybrid Assay and Library Screen

Two different systems were used for the two-hybrid analysis: The Gal4 system used a Gal4 DNA-binding domain in the bait constructs (vector = pAS1-CYH2) expressed in strain Y190 (Table 1). The LexA system used a LexA DNA-binding domain [pBTM116 or pBTM-C(x)] in strain L40. Log phase cells were cotransformed with construct pairs (except in the case of the library screen) or were transformed with appropriate individual plasmids by the lithium acetate method. Transformants were grown for 2–4 d at 30°C before filter lift assays were performed for detection of β-galactosidase activity (Bartel *et al.*, 1993).

For the library screen, a library containing three reading frames (Y2HL-C1, Y2HL-C2, and Y2HL-C3) (James *et al.*, 1996) was transformed into Y190 containing pAS1-SMY1 effectively as described by Firmenich and Redding (1993). To further enhance the transformation efficiency, library DNA was added with sheared carrier DNA, which was prepared according to Golemis *et al.* (1996). The transformation mix was shaken for 30 min at 30°C, and DMSO was added to a final concentration of 10%. The mix was heat shocked for 15 min at 42°C and incubated overnight at room temperature before plating. Plates contained 30 mM 3-amino-triazole (Sigma, St. Louis, MO) in SC medium lacking leucine and tryptophan. Colonies were grown 3–7 d at 30°C before filter lift assays were performed. A total

Table 2. Two-hybrid Constructs Used in This Study

Construct name	*SMY1* or *MYO2* fragment	Fragment source[a]	Vector used[a]
pBTM-SMY1	*SMY1* (*Sal*I[b]-*Pst*I)	YEpSMY1-26	pBTM116 (*Bam*HI[b]-*Pst*I)
pAS1-SMY1	*SMY1* (*Nco*I[c]-*Sna*bI)	YEpSMY1-26[c]	pAS1-CYH2 (*Nco*I-*Bam*HI[b])
pACT-SMY1	*SMY1* (*Xba*I[b]-*Sna*bI)	YEpSMY1-26	pACTII (*Bgl*II[b])
pGAD-SMY1 (D1)	*SMY1* (*Xba*I[b]-*Sna*bI)	YEpSMY1-26	pGAD-C1 (*Sma*I)
D2	*SMY1* (*Dra*I-*Pst*I)	pKS$^-$D9	pGAD-C1 (*Sma*I-*Pst*I)
D3[d]	*SMY1* (*Dra*I-*Sca*I)	pKS$^-$D9	pGAD-C1 (*Sma*I)
D4[d]	*SMY1* (*Xba*I[b]-*Dra*I)	YEpSMY1-26	pGAD-C1 (*Sma*I)
D5[e]	*SMY1* (*Dra*I-*Sst*I[b]-*Pst*I[b])	D2	pGAD-C1 (*Sma*I-*Pst*I[b])
D6	*SMY1* (*Ssp*I-*Pst*I)	pKS$^-$D9	pGAD-C2 (*Sma*I-*Pst*I)
D7	*SMY1* (*Sst*I[b]-*Pst*I)	D6	pGAD-C3 (*Bam*HI[b]-*Pst*I)
D9[f]	*SMY1* (*Ssp*I-*Hin*P1I-*Pst*I)	D6	pGAD-C1 (*Cla*I-*Pst*I)
pBTM-MYO2[g]	*MYO2* (*Bam*HI-*Pst*I)		pBTM116
pACT-MYO2	*MYO2* (*Bam*HI-*Nae*I)	pBTM-MYO2	pACTII (*Bam*HI-*EcoRI*[b])
Myo2R	*MYO2* (*Bam*HI-*Pst*I)	pBTM-MYO2	pGAD-C2 (*Bam*HI-*Pst*I)
M1	*Bgl*II digest and religation	pGAD-MYO2	
M2	*MYO2* (*Bgl*II-*Pst*I)	pGAD-MYO2	pGAD-C1 (*Bam*HI-*Pst*I)
M3[h]	*MYO2* (*Eco*RI[i]-*Eco*RV)	M2	pGAD-C1 (*Eco*RI-*Sma*I) pBTM-C1 (*Eco*RI-*Sma*I)
M4[h]	*MYO2* (*Eco*RV-*Pst*I)	M2	pGAD-C1 (*Sma*I-*Pst*I)
	MYO2 (*Eco*RI[i]-*Pst*I)	M2	pBTM116 (*Eco*RI-*Pst*I)
M73	*Afl*II digest and religation	pBTM-MYO2	
M76	*Afl*II digest and religation stop codon created at ligation	pBTM-MYO2	
pBTM-M11	*myo2-2* (*Spe*I-*Afl*II)	pNLC10	pBTM-MYO2 (*Spe*I-*Afl*II)
M2-2	*myo2-2* (*Bgl*II-*Pst*I-*Sal*I)	pNLC10	pGAD-C1 (*Bam*HI-*Sal*I)
pBTM-MYO4[g]			pBTM116

[a] Vectors and fragment sources are described in MATERIALS AND METHODS.

[b] Blunted with klenow fragment or T4 DNA polymerase.

[c] A 10-bp *Nco*I-*Xba*I polylinker was used.

[d] A short stretch of vector sequence occurs before stop codon is encountered.

[e] Construct D5 was made by inserting the fragment into the vector, digesting with *Sst*I and *Pst*I, blunting, and religating.

[f] The fragment for D9 was isolated and digested with *Hin*P1I before ligation into the receiving vector.

[g] pBTM-MYO2 and pBTM-MYO4, kindly provided by Ralf Jansen, contain the MYO2 tail fragment bp 3360–5306 and the equivalent fragment of the Myo4p tail.

[h] Constructs M3 and M4 were made in both pGAD-C(*x*) and pBTM-C(*x*) vectors in an attempt to increase their expression levels (see MATERIALS AND METHODS).

[i] *Eco*RI site is located in the MCS of pGAD-C1.

of 1.5 million transformants, each from Y2HL-C1 and Y2HL-C3, and 0.5 million from Y2HL-C2 were screened.

The colony filter lift assay was performed as previously described (Bartel *et al.*, 1993). In every assay, pairs of constructs previously shown to interact were used as positive controls, combinations that do not interact were used as negative controls, and individual constructs were tested for self-activation. When known pairs were tested, 50–1000 individual transformants were assayed from each of three or more independent transformation procedures. In all cases, color development was assessed at 3 h. (Little further change was observed in up to 18 h.)

Levels of fusion protein were checked for selected two-hybrid constructs by Western blotting. In all cases in which the presence of fusion protein could not be confirmed by two-hybrid analysis and/or Western blotting, cloning junctions were sequenced to confirm that the insert was in frame. In general, fusion proteins with smaller fragments of Myo2p or Smy1p appeared less abundant: Myo2R, M73, and M76 (Figure 1B) were much more abundant than endogenous Myo2p, M2 was much less abundant than endogenous Myo2p, and M3 and M4 could not be detected with the polyclonal antibody against Myo2p (but were detected in trace amounts using a LexA antibody). The mutant *myo2–2* fragment M2–2 was more abundant than the equivalent wild-type fragment M2 (Figure 1B), whereas the expression level of the other *myo2–2* fragment (M11) was roughly equivalent to the comparable wild-type construct Myo2R. In the case of Smy1p (Figure 1A), D5 protein was much more abundant than endogenous Smy1p, but D7 and D9 were not detected. In all cases in which proteins were detected by Western analysis, fragments were of the expected size.

Immunofluorescence Microscopy and Western Blotting

Growth conditions, cell preparations, antibody, and antibody incubations were as previously described (Lillie and Brown, 1994). Both Myo2p and Smy1p antibodies are polyclonal and directed against tail domains only (Lillie and Brown, 1994). For endogenous Myo2p (and myo2–2p) and Smy1p immunolocalization, affinity-purified antibody was used at 1:25 and 1:20, respectively. When Smy1p was

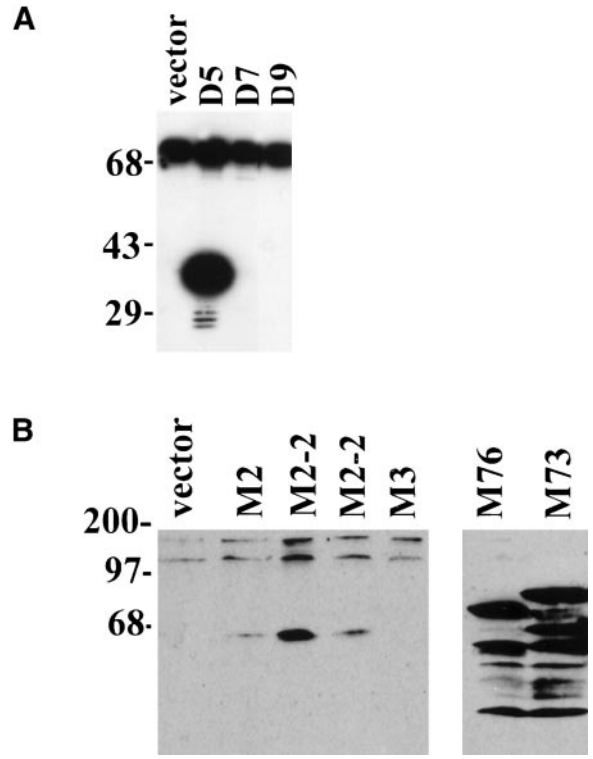

Figure 1. Western analysis of selected two-hybrid constructs. (A) Protein expression of *SMY1* fragments inserted into pGAD-C(*x*). The blot was probed with affinity-purified polyclonal anti-Smy1p. The ~70-kDa band is endogenous Smy1p. (B) Protein expression of *MYO2* and *myo2–2* fragments. M2, M2–2, and M3 fragments are inserted into pGAD-C(*x*), whereas M73 and M76 are in pBTM116. The ~180-kDa band is endogenous Myo2p. Because of their strong expression, a shorter time exposure (25 s instead of 2 min) is shown for M73 and M76. Expression levels and constructs are described in MATERIALS AND METHODS. Constructs also are described in Table 2 and illustrated in Figure 2.

overexpressed, antibody dilutions of 1:60–1:150 were used. Secondary antibody was 1:200 fluoroscein isothiocyanate–conjugated goat anti-rabbit immunoglobulin G (IgG; Sigma).

Specimens were viewed with an Orthoplan fluorescence microscope, and digital images were collected with a Sony DKC 5000 video–charge-coupled device camera. Images were optimized using Adobe (San Jose, CA) Photoshop.

For the Western blots, a total of ~2×10^8 log phase cells were collected by centrifugation. The pellet was transferred to microcentrifuge tubes and resuspended in 200 μl of 5% trichloroacetic acid. Glass beads (0.5 mm) were added up to the meniscus, and samples were vortexed 1 min and then microfuged 5 min at high speed (~12,000 × *g*) at 4°C. Supernatant and beads were removed, and the pellet was washed with 1 ml water. The pellet was resuspended in 50 μl 2× SDS solubilizing buffer + 50 μl PBS + complete protease inhibitors (Boehringer Mannheim, Indianapolis, IN). The suspension was boiled 3 min, vortexed 1 min, and microfuged 30 s. Thirty to 50 μl of sample were loaded onto 5 or 7% SDS-polyacrylamide minigels (Laemmli, 1970).

Proteins were blotted onto nitrocellulose as previously described (Lillie and Brown, 1987). Blots were blocked with 5% milk/PBS and incubated at room temperature with 1:100–200 dilutions of affinity-purified polyclonal anti-Myo2p or anti-Smy1p. The monoclonal antibody against LexA (Clontech) was used at a concentration of 10 ng/ml. As appropriate, either horseradish peroxidase–conjugated goat anti-rabbit IgG (Bio-Rad, Hercules, CA) or horseradish peroxidase–conjugated goat anti-mouse IgG (Sigma) were used as secondary antibodies, and a chemiluminescence kit (Boehringer Mannheim) was used for detection.

Table 3. Smy1p interacts with Myo2p in two different two-hybrid systems, independent of vectors used

	2-Hybrid interaction
LexA System constructs[a]	
pBTM-SMY1[b] + pACT-MYO2[d]	++[d]
pBTM-SMY1 + Myo2R (pGAD)[e]	++
pBTM-MYO2 + pACT-SMY1	++
pBTM-MYO2 + pGAD-SMY1[e]	++
Gal4 system constructs[a]	
pAS1-SMY1 + pACT-MYO2	++
pAS1-SMY1 + pGAD-MYO2	−

[a] All fusion proteins were detected by Western analysis.

[b] The N terminus of all Smy1p fusions is truncated by 18 amino acids, except for pBTM-SMY1, which is truncated by 43 amino acids.

[c] The Myo2p fusions begin with the coiled-coil domain (amino acid 927) and continue to the C terminus of the tail.

[d] ++, robust two-hybrid interaction which corresponds to 15-fold greater activity than the background activity determined with the individual plasmids; −, no two-hybrid interaction detected.

[e] These pairs were tested in a liquid beta-galactosidase assay (Kaiser *et al.*, 1994) and found to have 15-fold greater activity than the background activity determined with the individual plasmids.

RESULTS

Smy1p Interacts with Myo2p in the Two-Hybrid Assay

Smy1p and Myo2p display an intimate relationship, as determined by colocalization and genetic interactions (Lillie and Brown, 1992, 1994, 1998). This prompted us to ask whether they might physically interact. Using the two-hybrid assay, we discovered that Smy1p does indeed interact with Myo2p. The two-hybrid interaction between Smy1p and the Myo2p tail is reproducible, vector independent, and detectable in both the LexA and Gal4 assay systems (Table 3). The one exception occurs under circumstances in which expression is low [pGAD-C(*x*) vectors have a weak promoter; see MATERIALS AND METHODS], and the assay is less sensitive (the Y190 strain used in the Gal4 system has a reduced number of binding sites; Table 1).

In an attempt to identify other interacting proteins we used *SMY1* bait to screen a two-hybrid yeast genomic library. So far, we have used a library created in the pGAD-C(*x*) vector series (James *et al.*, 1996) and screened using the Gal4 system. The screen was completed to a confidence of 99% (James *et al.*, 1996; see MATERIALS AND METHODS) and 30+ candidates were obtained. However, on the basis of their ability to self-activate or on the loss of the positive interaction after plasmid recovery and retransformation, all candidates were determined to be false-positives. (Given the combination of vector and assay system, we did not expect

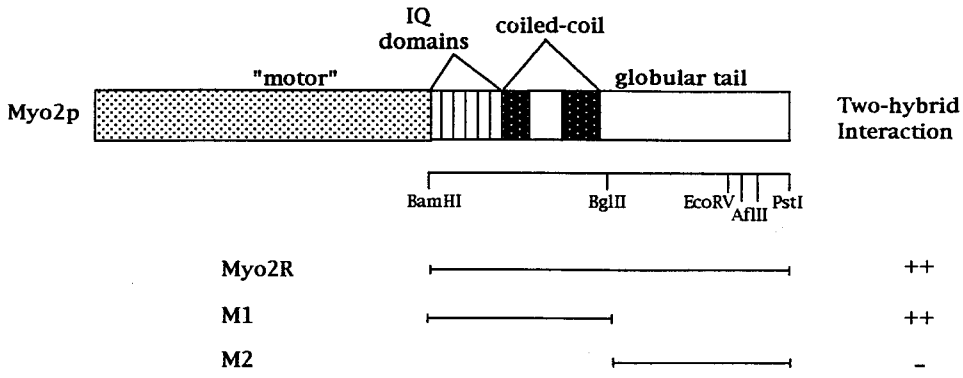

Figure 2. Myo2p forms homodimers. pBTM-MYO2 (same insert as MYO2R) was tested for two-hybrid interaction with three pGAD-C(x) constructs: MYO2R, M1, and M2. The interaction of M1 but not M2 suggests the coiled-coil domain is necessary for dimerization.

to pull Myo2p; see Table 3.) We therefore have no evidence that Smy1p interacts with any protein other than Myo2p. A useful conclusion that we can draw is that the *SMY1* bait is not promiscuous (i.e., does not interact nonspecifically with a large number of irrelevant proteins).

Smy1p and the tail of Myo2p also were tested for two-hybrid interactions with various other proteins of interest. For example, we tested α- and β-tubulin (plasmids obtained from K. Richards and D. Botstein, Stanford University, Stanford, CA), actin (K. Schwartz and D. Botstein), and Sec2p and Sec4p (obtained from R. Collins and P. Novick [Yale University, New Haven, CT] and included because of synthetic lethal interactions reported by Lillie and Brown, 1998). Neither Smy1p nor the Myo2p tail was found to interact with any of these proteins. We also tested both the Myo2p tail and Smy1p with the Myo4p tail (plasmid obtained from R. Jansen, University of Heidelberg, Heidelberg, Germany) and did not observe a two-hybrid interaction. Myo4p shares homology with Myo2p even outside the motor domain (Haarer *et al.*, 1994) and is the only other class V myosin found in yeast. These characteristics made Myo4p a useful negative control for both the Smy1p-Myo2p interaction, and the Myo2p-Myo2p interaction discussed below.

A Myo2p–Myo2p Interaction Detected by Two-Hybrid Analysis

We have determined that Myo2p interacts with itself, and the interaction can be mapped to the coiled-coil domain (Figure 2). When full-length tails were tested against one another, a positive two-hybrid interaction was detected. We then tested either the coiled-coil (M1) or the globular portion of the tail (M2) and found that the former but not the latter retained the ability to interact. To ask about the specificity of this interaction, we tested our constructs (Myo2R, M1, and M2 in Figure 2) against the analogous portion of Myo4p tail and found that none of them interacted. Myo4p is an optimal control because it is another yeast class V myosin. We conclude that Myo2p dimerizes via a coiled-coil interaction, as has been shown to be the case for another class V myosin (Cheney *et al.*, 1993).

Because Smy1p also contains a predicted coiled-coil domain, we tested Smy1p for two-hybrid interactions with itself. Only an extremely weak positive signal was obtained. Thus, two-hybrid analysis does not provide strong evidence for homodimerization of Smy1p.

Domain Mapping of the Smy1p–Myo2p Two-Hybrid Interaction

Based on sequence comparisons, Smy1p is one of the most divergent members of the kinesin superfamily (Lillie and Brown, 1992; Goldstein, 1993). Nonetheless, it is predicted to have the same general layout as conventional kinesin: a conserved "motor"/head domain, and a tail comprising a putative coiled-coil domain, followed by a globular domain (illustrated in Figure 3A). To determine which domain is responsible for the two-hybrid interaction with Myo2p, we created a series of *SMY1* two-hybrid constructs (Table 2). As described below, these constructs allowed us to narrow down the two-hybrid myosin-binding region (MBR) to 69 amino acids within the globular tail domain of Smy1p (Figure 3A).

We first asked whether the MBR was located in the head or the tail of Smy1p. Comparison of construct D4 to D2 (Figure 3A) demonstrates that the Smy1p tail interacts with the Myo2p tail, but that the Smy1p head does not. Next, we asked which domain of the Smy1p tail was necessary for the interaction. We found that the coiled-coil domain of Smy1p (D5) does not interact with the Myo2p tail. Western blotting (Figure 1A) shows that D5 is strongly expressed, confirming that its lack of interaction is not due to a lack of fusion protein. On the other hand, the globular portion of the tail did show an interaction (D6 and D7). The fact that D6 gives a stronger two-hybrid signal than D7 may indicate that the full MBR includes a sequence upstream of D7. Alternatively, the weaker signal of D7 may be a result of a lower level of fusion protein (see MATERIALS AND METHODS). A comparison of results with D2 and D3 indicates that the C-terminal 9 amino acids are dispensable, whereas further truncation (compare D3 with D5) abolishes the interaction. This places the MBR mostly or entirely in a 69-amino acid region of the Smy1p tail spanning amino acid 578 to amino acid 647. Further subdivision of this region gives fragments (e.g., construct D9) that produce a very weak positive signal, suggesting that these contain only a portion of the MBR.

We have also attempted to determine the putative Smy1p-binding site on the Myo2p tail. Like other class V myosins, Myo2p contains a conserved head/motor domain, IQ domains, and a tail comprising coiled-coil and globular domains (Figure 3B). Testing a series of truncated Myo2p two-hybrid constructs (Table 2) allowed us to narrow the Smy1p-binding region down to the globular portion of the Myo2p

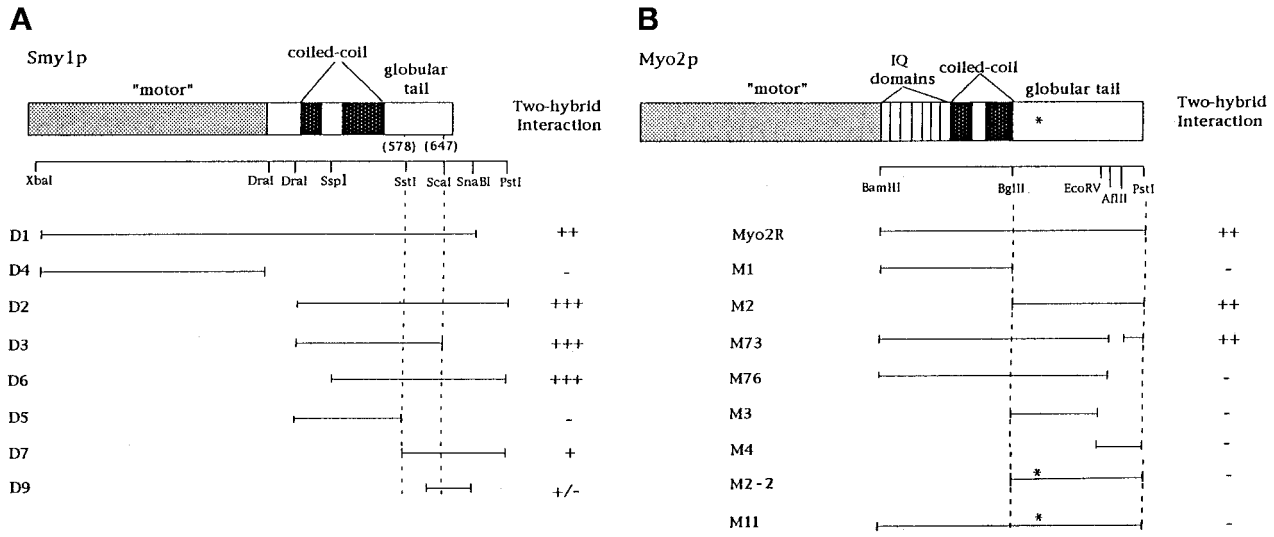

Figure 3. Domain mapping of the Smy1p–Myo2p interaction by two-hybrid analysis. (A) *SMY1* fragments were inserted into pGAD-C(*x*) and tested for two-hybrid interaction with pBTM-*MYO2*. (B) Fragments of *MYO2* and *myo2–2* inserted into pGAD-C(*x*), Myo2R, M1, M2, M3, M4, and M2–2 were tested for interaction with pBTM-SMY1(D1). M73, M76, and M11 were constructed in pBTM116 and tested for interaction with pGAD-SMY1. M3 and M4 fragments were also inserted into pBTM-C1 and pBTM116, respectively. The asterisk indicates the G1248 to D1248 mutation of myo2–2p (Catlett and Weisman, 1998).

tail (cf. M1 and M2, Figure 3B) (Note the results in Figure 2 provide a positive control for the M1 construct by demonstrating that it is capable of giving a two-hybrid reaction with a different partner). We have not been able to specify the location of the Smy1-binding site more exactly than this, although a comparison of M73 and M76 (Figure 3B) suggests that sequence at the C terminus of the globular tail domain is involved. However, if the Smy1p-binding site were restricted to the end of the C terminus, we would predict that M4 (but not M3, Figure 3B) would be positive by two-hybrid analysis. Because this is not the case, some other portion of the globular tail may be involved as well, or there may be too little M4 fusion protein present (see MATERIALS AND METHODS).

The MBR of Smy1p Is Required for Functional Rescue of the myo2–66 Mutant

Smy1p when overexpressed, partially overcomes the defect in polarized growth in the *myo2–66* mutant, although the mechanism of suppression has remained elusive. Our new observation that Smy1p and Myo2p display two-hybrid interaction would seem to provide a physical basis for the suppression. Therefore, we have asked whether the 69-amino acid MBR of the Smy1p tail is required for suppression of the *myo2–66* mutant phenotype.

High-copy-number *SMY1* constructs containing or lacking the MBR were tested for their ability to overcome the temperature-sensitive growth defect of the *myo2–66* mutant (Figure 4A). The strain expressing the truncated Smy1p protein $Smy1p_{578}$ (missing the MBR) was unable to grow at restrictive temperature. In contrast, $Smy1p_{647}$ (truncated downstream of the MBR), like the full-length control Smy1p, was able to rescue the *myo2–66* mutant at restrictive temperature, indicating that the MBR is required for suppression. Western blotting reveals approximately equal levels of proteins of the predicted sizes from all three constructs (Figure 4B). On the basis of the two-hybrid and the suppression data, we postulate that Smy1p must bind to Myo2p in order to overcome the *myo2–66* mutant phenotype.

The MBR of Smy1p Is Required for the Normal Localization of Smy1p

To test the importance of the MBR for Smy1p localization, we used the same constructs, $YEpSMY1_{578}$ and $YEpSMY1_{647}$, that were used above, transformed into a *SMY1* null (*smy1*Δ) strain. As expected, no Smy1p was seen in cells with vector alone (Figure 5a), whereas distinct caps were seen in cells carrying full-length *SMY1* (Figure 5b). Cells expressing $Smy1p_{647}$ also had distinct caps (Figure 5c). However, in cells expressing $Smy1p_{578}$ and therefore lacking the MBR, Smy1p was diffuse throughout the cytoplasm (Figure 5d). These results indicate that the MBR is necessary not only to overcome the *myo2–66* mutant phenotype, but also for the normal localization of Smy1p.

Localization of Smy1p in the myo2–2 Mutant

A new mutant allele of *MYO2* (*myo2–2*) has been isolated and characterized by Catlett and Weisman (1998). The *myo2–2* mutation lies in a region encoding the globular portion of the tail (Gly1248 to Asp1248). In contrast, the *myo2–66* mutation is found in a region encoding the actin-binding face (Lillie and Brown, 1994). Catlett and Weisman (1998) have found that myo2–2p does not localize normally, even though the actin cytoskeleton appears normal and polarized growth seems unaffected. In contrast, myo2–66p

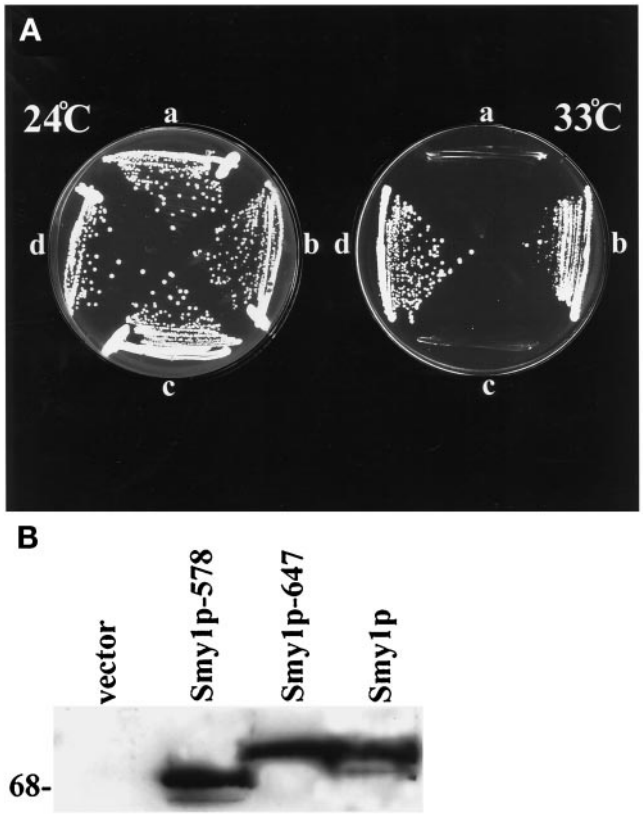

Figure 4. The MBR of Smy1p is necessary to overcome the temperature-sensitive growth defect of the *myo2–66* mutant. (A) The yeast strain SLY88 carrying the *myo2–66* mutation was transformed with high-copy-number plasmid vectors YEp352 (a), YEpSMY1$_{647}$ (b), YEpSMY1$_{578}$ (missing the MBR) (c), and YEpSMY1–26 (full length) (d) . Transformants were grown at permissive temperature (24°C) and at restrictive temperature (33°C) for 3–4 d on selective medium. The *myo2–66* mutant cells overexpressing full-length Smy1p (656 amino acids) (d) and Smy1p truncated at amino acid 647 (b) were able to grow at 33°C, but those without overexpressed Smy1p (a) or with Smy1p truncated at amino acid 578 (c) were not. (B) Western analysis of vector YEp352, YEpSMY1$_{578}$ (missing the MBR), YEpSMY1$_{647}$, and YEpSMY1 (full length) in the *smy1*Δ (null) strain SLY86 demonstrates nearly equivalent levels of protein expression. Constructs are described in MATERIALS AND METHODS.

fails to localize only at restrictive temperature, when actin organization and polarized growth also are disrupted. The *myo2–2* mutant thus affords us an opportunity to look at the relation between Smy1p and Myo2p localization without concomitantly perturbing the actin. We have used this mutant allele to ask three questions. First, given that myo2–2p is not localized normally despite normal actin localization, does Smy1p localize? Second, given that overexpression of Smy1p enhances Myo2p localization in wild-type cells and restores it in *myo2–66* cells (Lillie and Brown, 1994), does overexpression of Smy1p also restore the localization of myo2–2p? Third, if so, do the effects of overexpression of Smy1p depend on the MBR in the Smy1p tail?

The results of these experiments are shown in Figure 6. Like myo2–2p (Figure 6c), Smy1p is not detectable at sites of active growth in the *myo2–2* mutant (Figure 6d). To ask whether there might be some residual localization of these proteins that is below detectable limits, we turned to the fluorescent probe CY3 conjugated to secondary antibody (Ayscough and Drubin, 1998). This fluorophore has been used to detect Myo2p localization that had been undetectable with fluorescein isothiocyanate (Ayscough, personal communication). However, the localization of Smy1p and myo2–2p remained undetectable in the *myo2–2* strain when CY3 was used.

In answer to our second question, we found that overexpression of Smy1p can partially restore the localization of myo2–2p to the bud tip (Figure 6e). However, normal Smy1p localization is still not detectable (Figure 6f). It is possible that a weak Smy1p localization signal at the bud tip might be masked by the increased cytoplasmic signal when Smy1p is overexpressed. We have observed in wild-type *MYO2* cells that this problem can be overcome by examining the less brightly stained cells in the population and by varying the concentration of antibody. However, these approaches did not reveal Smy1p caps in the Smy1p-overexpressing *myo2–2* mutant. Two-hybrid analysis failed to show an interaction of Smy1p with the tail of myo2–2p, even though this construct was expressed approximately as well as the parallel wild-type construct (Figures 1B and 3B). Thus, a reduction in the affinity of myo2–2p for Smy1p probably contributes to the reduced localizations we have observed.

The ability of overexpressed Smy1p to restore the localization of myo2–2p depends on the presence of the MBR in the Smy1p tail. Overexpression of Smy1p$_{578}$ (missing the MBR) is not capable of restoring the myo2–2p localization (Figure 6*g*), whereas overexpression of wild-type Smy1p can (Figure 6*e*). This demonstrates that the MBR of the Smy1p tail is necessary for Smy1p to exert its influence not only on *myo2–66*, as discussed above, but also on the *myo2–2* allele. Thus, Smy1p is not merely stabilizing an altered domain of Myo2p, because these alleles alter different domains. Instead, it is enhancing the function of Myo2p in a way that compensates for two different defects.

DISCUSSION

Two-Hybrid Interaction between Smy1p and Myo2p

SMY1 was originally isolated as a multicopy suppressor of *myo2–66*, which encodes a defective class V myosin (Lillie and Brown, 1992). Our subsequent studies have provided strong support for the significance of this interaction (see INTRODUCTION), but it was not obvious how a myosin- and a kinesin-related protein would cooperate in a common function. One hypothesis was that Smy1p could compensate for the defective myosin by transporting the cargo via microtubules instead. Although the spatial and temporal arrangement of microtubules is conducive to this hypothesis (Kilmartin and Adams, 1984), we have determined that microtubules are not required for Smy1p localization or for the rescue of the *myo2–66* mutant phenotype (Lillie and Brown, 1998).

An alternative hypothesis is that Smy1p and Myo2p may cooperate through some form of a physical interaction. In this article we have presented evidence that this is in fact the case. We have observed a two-hybrid interaction between Smy1p and Myo2p, which we have confirmed in two differ-

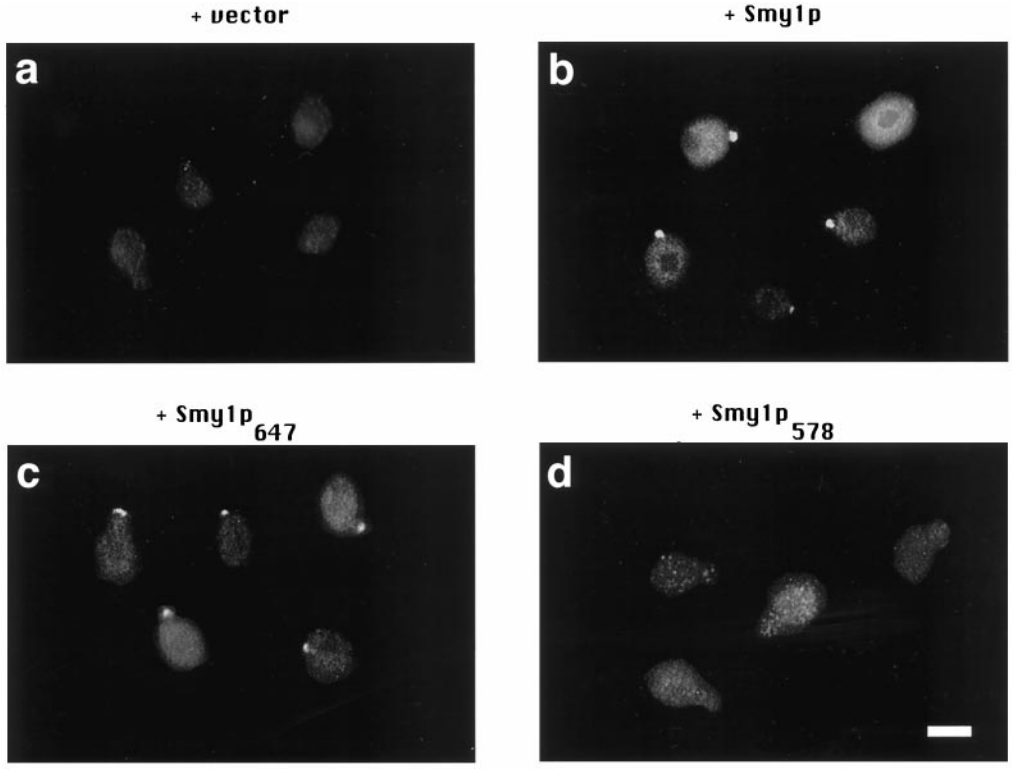

Figure 5. The MBR of Smy1p is necessary for localization of Smy1p. The *smy1*Δ (null) strain SLY86 was transformed with high-copy-number plasmids YEp352 (*URA3*) (a), YEpSMY1–26 (b), YEpSMY1$_{647}$ (c), and YEpSMY1$_{578}$ (missing the MBR) (d). Full-length Smy1p (b) and Smy1p$_{647}$ (c) localize normally, whereas Smy1p$_{578}$, missing the MBR (d), does not localize but is diffuse throughout the cytoplasm. See Figure 4B for expression levels of these constructs. Bar, 5 μm.

ent two-hybrid systems. We have also swapped vectors and have demonstrated by testing with library and known proteins that neither the Myo2p nor Smy1p two-hybrid protein is promiscuous. These proteins associate via their globular tail domains, and the site of two-hybrid interaction in Smy1p has been further mapped to a 69-amino acid region that we refer to as the MBR. Because we have shown that the MBR is required for Smy1p to suppress the *myo2–66* mutant phenotype, we propose that Smy1p corrects the myosin defect via this physical interaction.

We chose to look for an interaction between Smy1p and Myo2p using two-hybrid analysis because it is performed in vivo and allows very sensitive detection (Phizicky and Fields, 1995). To investigate the possibility that some other protein(s) might contribute to the interaction, we have attempted to observe the interaction in vitro by coimmunoprecipitation and coaffinity purification, using proteins expressed in either yeast or bacteria. The attempts were unsuccessful, but there are good reasons to suspect that the association of Smy1p and Myo2p may be labile. First, the localization of these proteins to caps is easily disrupted (Lillie and Brown, 1994); thus, caps are not expected to survive cell lysis. Second, the localization is cell cycle dependent, suggesting that the association between Smy1p and Myo2p may be highly regulated. Therefore, we believe that the negative in vitro results in no way undermine our discovery that Smy1p and Myo2p associate, especially given the wealth of other supporting evidence provided in this and previous articles.

Localization Studies

We have also shown that the MBR is required for Smy1p to localize to the bud tip. Therefore, we propose that it localizes via its binding to Myo2p. This fits with our finding in *myo2–2* cells that Smy1p does not reside independently at the bud tip but requires Myo2p. There are indications that some activity, in addition to Myo2p binding, may be involved in Smy1p localization. For example, Ayscough *et al.* (1997) have shown that upon treatment with latrunculin-A to disrupt actin filaments, 20% of treated cells have weak but detectable Myo2p caps, but no Smy1p caps, using antibodies we provided. However, given the faintness of their Myo2p signal and the fact that Smy1p signal is less strong than Myo2p signal with our antibodies (Lillie and Brown, 1998), we believe that Smy1p localization would have been difficult to detect. A second indication is our finding that a "headless" Smy1p does not localize (Lillie and Brown, 1998). However, the deletion might have caused folding problems that interfere with other domains. Therefore, it remains possible that Smy1p localization is dependent only on Myo2p.

The localization of myo2–2p presents several puzzles. First, it is surprising that the myo2–2 mutant functions

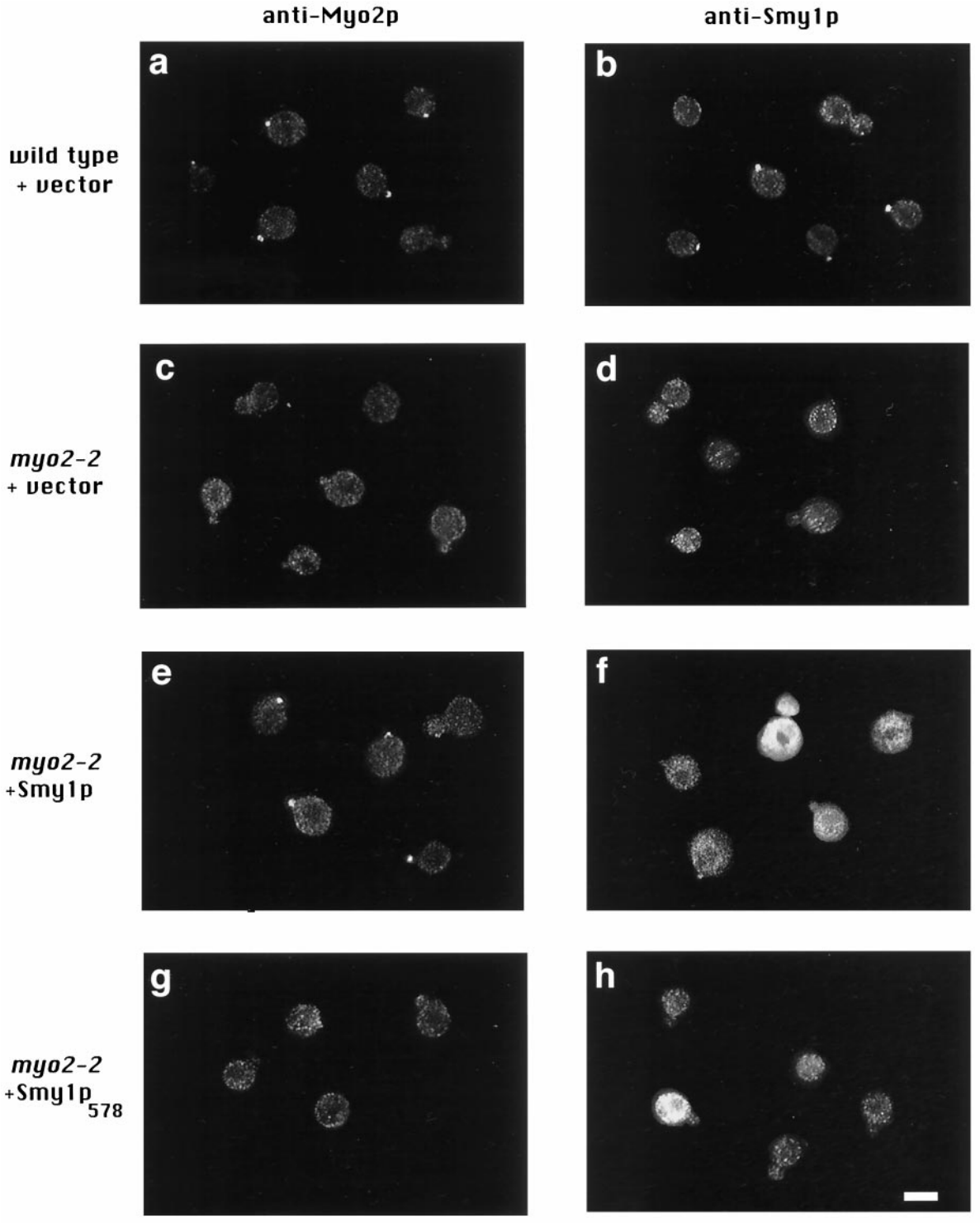

Figure 6. Localization of Smy1p and myo2–2p in the *myo2–2* mutant. (a and b) Localization of Myo2p (a) and Smy1p (b) in the wild-type strain LWY7213 carrying the high-copy-number vector YEp351 (*LEU2*). Localization of myo2–2p (c) and Smy1p (d) are not detected in the *myo2–2* mutant strain LWY2599–carrying vector. Localization of myo2–2p is detectable in LWY2599 when Smy1p is overexpressed from plasmid YEpSMY1–38 (e), but Smy1p localization is not, even in the cells with less background cytoplasmic staining (f). Neither myo2–2p nor Smy1p localization is detected when $Smy1p_{578}$ (missing the MBR) is overexpressed from the plasmid $YEpSMY1_{578}$-351 in LWY2599 (g and h). Constructs are described in MATERIALS AND METHODS under DNA Manipulations. Bar, 5 μm.

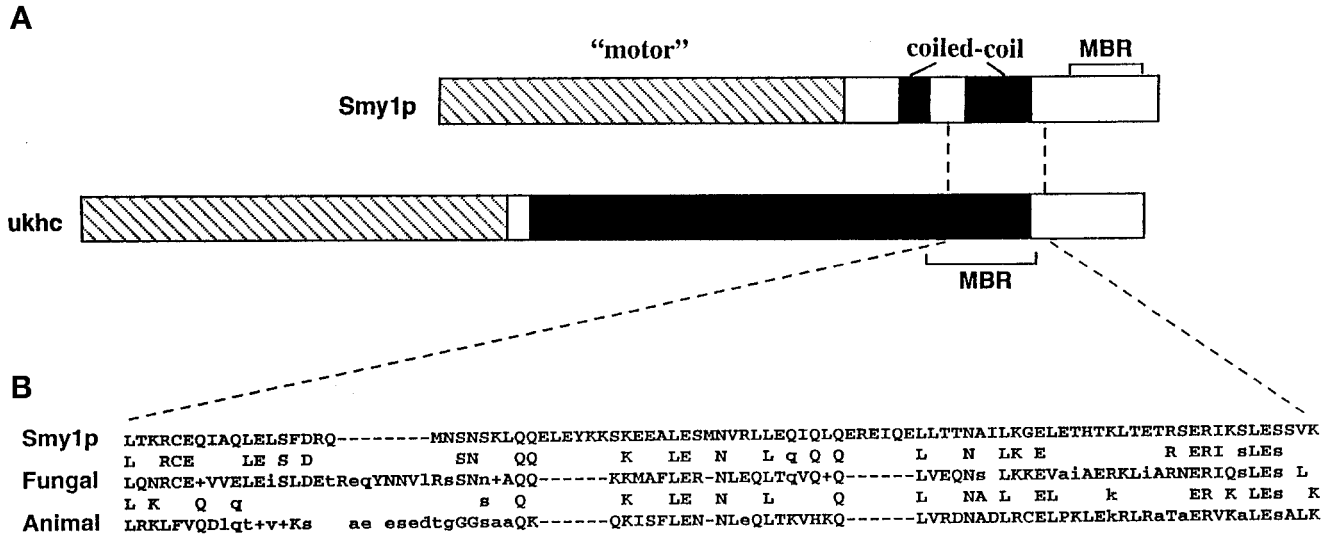

Figure 7. Comparison of Smy1p and mouse ubiquitous kinesin heavy chain (ukhc). (A) Cross-hatched boxes indicate the motor domains, black boxes the predicted coiled-coil domains, and brackets the myosin-binding regions (MBR) identified by two-hybrid analysis (this article; Huang *et al.*, 1999). The two proteins are aligned on the basis of a small region of sequence similarity near the ends of the coiled-coils. (B) Alignment of Smy1p (amino acids 468–559 Swiss Prot accession number P32364) with consensus sequences for animal and fungal kinesins. Hyphens indicate gaps that were introduced in the sequences, and + indicates conservation of a positively charged amino acid. This region of Smy1p also showed sequence similarity with the end of the coiled-coil region of lamin B (Swiss Prot accession number P14732. This alignment also revealed a couple of downstream clusters of serines that were spaced in a similar manner. Smy1p also shows a lesser degree of similarity to a variety of other coiled-coil proteins.

well in polarized growth despite having lost its polarized localization to sites of such growth. Second, it is surprising that excess Smy1p can restore myo2–2p localization despite the lack of two-hybrid interaction with the mutant protein. A possible explanation is that myo2–2p's loss in affinity for Smy1p may affect its retention at the bud tip but not its delivery there. (In addition, some loss in bud tip localization might result from the loss in vacuolar delivery.) For this explanation to work, we postulate that the loss of affinity is not total; there must be sufficient remaining affinity for Smy1p to have an effect on myo2–2p when overexpressed.

Smy1p Is Not Involved in Vacuole Transport by Myo2p

Myo2p has been implicated in polarized delivery, not only for bud growth but also for vacuolar inheritance (Hill *et al.*, 1996; Catlett and Weisman, 1998). Interestingly, Smy1p seems to play a role only in the former process. Catlett and Weisman (1998) have determined that both *myo2–66* and *myo2–2* mutants are defective in vacuole inheritance and that overexpression of Smy1p does not correct the defect in either case. Unlike the *myo2–66* mutation, they found that the *myo2–2* mutation does not affect polarized growth, nor is it synthetically lethal with deletion of *SMY1*. In addition, unlike *smy1*Δ *(SMY1* deletion) and *myo2–66* (Lillie and Brown, 1998), the *myo2–2* mutation is not synthetically lethal with two late secretory mutants, *sec2* or *sec4* (Catlett and Weisman, 1998). This fits with the idea that the Myo2p-Smy1p association plays a role at a late step of the secretory pathway that also involves Sec2p and Sec4p but has no role in vacuole inheritance. We infer from the above findings that the loss in affinity of myo2–2p for Smy1p that we have observed is unrelated to its vacuolar delivery defect. Because the myo2–2p mutation introduces a charged amino acid into the globular tail of Myo2p, it may alter folding and interfere separately with the binding of vacuolar cargo and the association of Smy1p to this region of Myo2p.

Parallels with Other Organisms

No homologues of Smy1p have been found in other organisms, raising the issue of whether it is a kinesin-related protein that is uniquely specialized for interactions with a myosin. However, the recent findings of Huang *et al.* (1999) lead us to believe otherwise. These authors, using fragments of mouse myosin Va as bait in a two-hybrid screen, have found an interaction with the ubiquitous heavy chain of conventional kinesin. Thus, like us, they have found that the globular portion of a class V myosin tail can associate with the tail of a member of the kinesin superfamily. However, the extent of the similarities between the two kinesins is not clear. Although Smy1p has been classified as an "orphan" kinesin, it does share a small region of sequence similarity with conventional kinesin (Figure 7). What's more, this cor-

responds to the only region of conserved sequence between the tails of animal and fungal kinesins (Steinberg and Schliwa, 1995). These authors suggest that similarity in this region may be diagnostic of the conventional kinesin subfamily, because it is not shared by kinesin-related proteins in other subfamilies. When Smy1p and mouse ubiquitous kinesin are aligned using this region, it can be seen that the two-hybridizing regions are not superimposed (Figure 7). Despite this, we believe these kinesins may be associated with myosin Vs in a similar way. Each two-hybridizing region may only be part of the interaction site. For example, it can be seen in Figure 3A that we obtain a stronger two-hybrid interaction when more of the Smy1p tail is included in the bait, and the same might be true of the interaction reported by Huang *et al.* (1999).

Possible Function of a Kinesin–Myosin Interaction

Kuznetsov *et al.* (1992) have shown that a single vesicle/organelle can move along a microtubule and then switch to an actin filament. This and succeeding observations (for a review, see Brown, 1999) have led to the idea that microtubules are used for long-range transport, followed by local delivery on actin filaments. It would be desirable to coordinate the motors involved, so that one motor is turned off at the same time the other is turned on, to prevent the motors from working against each other. A physical interaction between motors would provide a reasonable way of mediating such regulation. We speculate that Smy1p may directly or indirectly induce a conformational change in Myo2p that enhances its interaction with actin and thus its localization. Such a mechanism could explain how Smy1p both rescues the mutant *myo2–66* (mutation in the actin-binding site) and restores localization of the tail mutant *myo2–2*. In other systems, the switch can presumably be flipped in the other direction, so that the myosin is turned off when the kinesin is turned on. In our system, Smy1p may not even have motor activity (Lillie and Brown, 1998), and if it does, that activity is not required for suppression (Lillie and Brown, 1994). Regardless of whether some functions of Smy1p have been lost, we propose that its ability to upregulate Myo2p has been retained.

It will be interesting to learn from future studies precisely how Smy1p and Myo2p coordinate their actions. Nonetheless, the discovery that their behaviors are mediated by some form of a physical interaction adds a new dimension to the subject of molecular motors.

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REFERENCES

Ayscough, K., and Drubin, D. (1998). Immunofluorescence microscopy in yeast cells. In: Cell Biology: A Laboratory Handbook, vol. 2, ed. J. Celis, New York: Academic Press, 477–485.

Ayscough, K.R., Stryker, J., Pokala, N., Sanders, M., Crews, P., and Drubin, D.G. (1997). High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin-A. J. Cell Biol. *137*, 399–416.

Bartel, P.L., Chien, C.-T., Sternglanz, R., and Fields, S. (1993). Using the two-hybrid system to detect protein-protein interactions. In: Cellular Interactions in Development: A Practical Approach, ed. D.A. Hartley, Oxford: Oxford University Press, 153–179.

Brown, S.S. (1999). Cooperation between microtubule- and actin-based motor proteins. Annu. Rev. Cell Dev. Biol. *15*, 63–80.

Catlett, N.L., and Weisman, L.S. (1998). The terminal tail region of a yeast myosin-V mediates its attachment to vacuole membranes and sites of polarized growth. Proc. Natl. Acad. Sci. USA *95,* 14799–14804.

Cheney, R.E., O'Shea, M., Heuser, J., Coelho, M., Wolenski, J., Espreafico, E., Forscher, P., Larson, R., and Mooseker, M.S. (1993). Brain myosin-V is a two-headed unconventional myosin with motor activity. Cell *75*, 13–23.

Dagher, M.-C., and Filhol-Cochet, O. (1997). Making hybrids of two-hybrid systems. Biotechniques *22,* 916–922.

Firmenich, A., and Redding, K. (1993). An efficient procedure for multiple transformations of yeast in parallel. Biotechniques *14,* 713–718.

Goldstein, L.S.B. (1993). With apologies to Scheherazade: tails of 1001 kinesin motors. Annu. Rev. Genet. *27*, 319–351.

Golemis, E.A., Gyuris, J., and Brent, R. (1996). Interaction trap/two-hybrid system to identify interacting proteins. In: Current Protocols in Molecular Biology, vol. 2, ed. F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl, New York: John Wiley & Sons, 20.0.1–20.1.35.

Govindan, B., Bowser, R., and Novick, P. (1995). The role of Myo2, a yeast class V myosin, in vesicular transport. J. Cell Biol. *128*, 1055–1068.

Haarer, B.K., Petzold, A., Lillie, S.H., and Brown, S.S. (1994). Identification of *MYO4*, a second class V myosin gene in yeast. J. Cell Sci. *107*, 1055–1064.

Hill, J.E., Meyers, A.M., Koerner, T.J., and Tzagoloff, A. (1986). Yeast/*E. coli* shuttle vectors with multiple unique restriction sites. Yeast *2*, 163–167.

Hill, K.L., Catlett, N.L., and Weisman, L.S. (1996). Actin and myosin function in directed vacuole movement during cell division in *Saccharomyces cerevisiae*. J. Cell Biol. *135*, 1535–1549.

Huang, J.-D., Brady, S.T., Richards, B.W., Stenoien, D., Resau, J.H., Copeland, N.G., and Jenkins, N.J. (1999). Direct interaction of microtubule- and actin-based transport motors. Nature *397*, 267–270.

James, P., Halladay, J., and Craig, E.A (1996). Genomic libraries in a host strain designed for highly efficient two-hybrid selection in yeast. Genetics *144*, 1425–1436.

Johnston, G.C., Prendergast, J., and Singer, R.A. (1991). The *Saccharomyces cerevisiae MYO2* gene encodes an essential myosin for vectorial transport of vesicles. J. Cell Biol. *113*, 539–551.

Kaiser, C., Michaelis, S., and Mitchell, A. (1994). Methods in Yeast Genetics, Plainview, NY: Cold Spring Harbor Laboratory Press.

Kilmartin, J.V., and Adams, A.E.M. (1984). Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. J. Cell Biol. *98*, 922–933.

Kuznetsov, S.A., Langford, G.M., and Weiss, D.G.(1992). Actin-dependent movement in squid axoplasm. Nature *356*, 722–725.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature *227*, 680–685.

Lillie, S.H., and Brown, S.S. (1987). Artifactual immunofluorescent labeling in yeast, demonstrated by affinity purification of antibody. Yeast *3*, 63–70.

Lillie, S.H., and Brown, S.S. (1992). Suppression of a myosin defect by a kinesin-related gene. *Nature 356*, 358–361.

Lillie, S.H., and Brown, S.S. (1994). Immunofluorescence localization of the unconventional myosin, Myo2p, and the putative kinesin-related protein, Smy1p, to the same regions of polarized growth in *Saccharomyces cerevisiae*. J. Cell Biol. *125*, 825–842.

Lillie, S.H., and Brown, S.S. (1998). Smy1p, a kinesin-related protein that does not require microtubules. J. Cell Biol. *140*, 873–883.

Liu, H., and Bretscher, A. (1992). Characterization of *TPM1* disrupted yeast cells indicates an involvement of tropomyosin in directed vesicular transport. J. Cell Biol. *118*, 285–299.

Phizicky, E.M., and Fields, S. (1995). Protein-protein interactions: methods for detection and analysis. Microbiol. Rev. *59*, 94–123.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sherman, F., Fink, G.R., and Hicks, J.B. (1986). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Steinberg, G., and Schliwa, M. (1995). The *Neurospora* organelle motor: a distant relative of conventional kinesin with unconventional properties. Mol. Biol. Cell *6*, 1605–1618.