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Candida albicans INT1-Induced Filamentation in *Saccharomyces cerevisiae* Depends on Sla2p

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The *Candida albicans INT1* gene is important for hyphal morphogenesis, adherence, and virulence (C. Gale, C. Bendel, M. McClellan, M. Hauser, J. M. Becker, J. Berman, and M. Hostetter, Science 279:1355–1358, 1998). The ability to switch between yeast and hyphal morphologies is an important virulence factor in this fungal pathogen. When *INT1* is expressed in *Saccharomyces cerevisiae*, cells grow with a filamentous morphology that we exploited to gain insights into how *C. albicans* regulates hyphal growth. In *S. cerevisiae*, *INT1*-induced filamentous growth was affected by a small subset of actin mutations and a limited set of actin-interacting proteins including Sla2p, an *S. cerevisiae* protein with similarity in its C terminus to mouse talin. Interestingly, while *SLA2* was required for *INT1*-induced filamentous growth, it was not required for polarized growth in response to several other conditions, suggesting that Sla2p is not required for polarized growth per se. The morphogenesis checkpoint, mediated by Swe1p, contributes to *INT1*-induced filamentous growth; however, epistasis analysis suggests that Sla2p and Swe1p contribute to *INT1*-induced filamentous growth through independent pathways. The *C. albicans SLA2* homolog (*CaSLA2*) complements *S. cerevisiae sla2*Δ mutants for growth at 37°C and *INT1*-induced filamentous growth. Furthermore, in a *C. albicans Casla2*/*Casla2* strain, hyphal growth did not occur in response to either nutrient deprivation or to potent stimuli, such as mammalian serum. Thus, through analysis of *INT1*-induced filamentous growth in *S. cerevisiae*, we have identified a *C. albicans* gene, *SLA2*, that is required for hyphal growth in *C. albicans*.

Candida albicans is the most prevalent fungal pathogen of humans. In addition to causing mucosal infections, such as thrush and vaginitis, in relatively healthy individuals, it causes life-threatening systemic infections in premature infants, surgical patients, chemotherapy patients, and other patients with weakened immune systems. Mortality from systemic infections approaches 30% despite appropriate therapy with the available antifungal agents (59). *C. albicans* grows in a number of morphologic forms, including ellipsoidal, yeast-form blastospores and filamentous forms that include elongated budding pseudohyphae and parallel-sided germ tubes that give rise to true hyphae (reviewed in reference 58). The ability of *C. albicans* to switch between these morphologies is correlated with its virulence (reviewed in references 16, 51, and 58). The transition from yeast to hyphal growth occurs in response to a broad range of environmental stimuli. Potent stimuli include one or more constituents of mammalian serum, the presence of specific compounds (such as *N*-acetylglucosamine), temperature of 37°C, and neutral pH (reviewed in reference 16). A slower filamentous growth response is induced by nutrient deprivation on solid media, such as Spider agar and milk-Tween agar (40).

Many genes that contribute to *C. albicans* morphogenesis have been identified. These include members of the mitogen-activated protein kinase (MAPK) cascade that is analogous to the MAPK cascade important for mating and pseudohyphal growth in *Saccharomyces cerevisiae* and members of the RAS-cyclic AMP (cAMP) signaling pathway, which also contributes to pseudohyphal growth in *S. cerevisiae* (13, 20). The MAPK and RAS-cAMP signaling pathways activate transcription factors, such as Cph1p and Efg1p, respectively (44). Other transcriptional activators and repressors, such as Tup1p, Rbf1p, Prr2p, and Czf1p, also contribute to *C. albicans* morphogenesis under some environmental conditions (reviewed in references 19 and 51). Loss of transcription factor function usually results in altered filamentation in response to a subset of the conditions that elicit filamentous growth (12, 20). The current working model is that there are several (≥3) independent pathways for triggering morphogenetic changes (12, 19). In addition, there is a significant amount of "cross-talk" between these pathways that may differ, depending upon the environmental stimuli present. Because the relationships between different elicitors, signaling pathways, and morphogenetic responses are complex and not well characterized, our goal is to improve our understanding of these complex relationships by determining the pathways and cellular processes regulated by the different morphogenetic signals.

Transcription factors, such as Efg1p and Tup1p, regulate cell morphogenesis, at least in part, by affecting the transcription of genes, such as those encoding cell wall components or cell wall maintenance functions (e.g., *ALS1, CHS2, HWP1*, and *HYR1* [7, 12, 31, 47, 62, 66]). Ultimately, morphogenesis signals must

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be executed by the actin cytoskeleton and the secretory vesicles which deliver cell wall material to the cell surface in regions of cell growth. During polarized growth, the actin cytoskeleton delivers most vesicles to the distal pole of the bud, while during isotropic growth, actin and secretory vesicles are observed distributed around the entire periphery of the growing daughter cell.

C. albicans INT1 was originally cloned by virtue of its limited homology to vertebrate leukocyte integrins (23), adhesion proteins that bind the extracellular matrix and induce morphologic changes in response to extracellular signals (32). In *C. albicans, INT1* is a virulence factor that contributes to the ability of the pathogen to adhere to epithelial cells (22). Like *CPH1* and many other genes, *INT1* is required for filamentous growth on milk-Tween and Spider medium, but is not required for filamentation on serum (22), which is considered to be a potent elicitor of the filamentation response. When expressed in *S. cerevisiae*, Int1p was detected on the cell surface, mediated adhesion to HeLa cell monolayers, and triggered the formation of highly polarized buds with a morphology similar to that of *C. albicans* germ tubes (22, 23; C. A. Gale, M. Gerami-Nejad, M. McClellan, M. S. Longtine, and J. Berman, submitted for publication). Unlike *S. cerevisiae* pseudohyphal growth, *INT1*-induced filamentous growth is independent of MAPK components, does not require specific genetic strain backgrounds, and occurs in either haploid or diploid cells (23). Based upon the integrin paradigm, these results are consistent with a model in which Int1p may be a *C. albicans* surface protein that responds to a subset of environmental stimuli and mediates a morphogenetic response to them.

In this study, we exploited the ability of *C. albicans INT1* to induce filamentous growth in *S. cerevisiae* to ask about the actin cytoskeleton components required for *INT1*-induced filamentous growth. We asked if processes that are well characterized in *S. cerevisiae* are important for *INT1*-induced filamentous growth in *S. cerevisiae* and then asked if they are also important for hyphal growth in *C. albicans*. We identified components of the actin cytoskeleton that affect *INT1*-induced filamentous growth, including a small subset of actin mutations and a limited set of actin-interacting proteins. One of these is Sla2p, the *S. cerevisiae* protein most closely related to mouse talin. Interestingly, while *SLA2* is required for *INT1*-induced filamentous growth, Sla2p is not required for *S. cerevisiae* to form highly polarized buds or mating projections, suggesting that Sla2p is not required for polarized growth per se. In addition, we determined that Sla2p does not trigger *INT1*-induced filamentous growth exclusively through the morphogenesis checkpoint mediated by Swe1p. In *S. cerevisiae, CaSLA2* complemented *S. cerevisiae sla2*Δ mutants for growth at 37°C and *INT1*-induced filamentous growth. In *C. albicans*, disruption of both *C. albicans SLA2* alleles resulted in strains that did not exhibit a hyphal growth response to mammalian serum or to nutrient deprivation. These results suggest that, in *C. albicans*, Sla2p is essential for hyphal growth in response to both potent and mild environmental stimuli.

MATERIALS AND METHODS

Plasmids, strains, culture conditions, and microscopy. Plasmids and strains used in this study are listed in Table 1. pCG110 and pCG108, which express *INT1* from the *GAL10* promoter, were derived from pCG01 by using marker swap plasmids pUL9 and pUT11, respectively (17). pYES2-CaSLA2 contains a chromosomal copy of *CaSLA2* obtained by PCR of fosmid 18B6 (obtained from the *Candida albicans* Mapping Project at the University of Minnesota, http://alces.med.umn.edu/candida/probeabout.html), using the following primers: forward, CGAGCTC(*Sac*I)CCCCCCCTAGCCCAATG(Start)AG, and reverse, GCTGC TATTGTTTGTTC, which contains a sequence downstream of the *Eco*RI site 3′ of the stop codon. The PCR fragment was digested with *Sac*I and *Eco*RI and cloned into *Sac*I- and *Eco*RI-digested pYES2 (Invitrogen, Inc.) to drive expression of *CaSLA2* from the *GAL1* promoter of *S. cerevisiae*. YJB3857 was generated from YEF473 (yML97) (9) transformed with pWA9 (*end4*::*HIS3*) (68). YJB5565 and YJB5566 are sister progeny from a cross of YJB3857 and M-1623 (YEF473 *cdc3-6*) obtained by nine successive backcrosses of a *cdc3-6* allele into the YEF473 strain background and was kindly provided by Mark Longtine, Oklahoma State University.

S. cerevisiae and *C. albicans* strains were grown in standard laboratory synthetic complete (SC) media with appropriate amino acid drop-outs (63). Media were supplemented after autoclaving with a carbon source as indicated in the text. *C. albicans ura3* mutant strains were grown on medium supplemented with 2 μg of uridine/ml. Standard transformation protocols were used for both *S. cerevisiae* (24) and *C. albicans* (25). YJB5565 and YJB5566 were derived from progeny of YJB5126 crossed to YJB3857.

To assay *INT1*-induced filamentous growth, *S. cerevisiae* cells were grown overnight in 2% glucose at room temperature. Cells were diluted fivefold into SC medium containing 1% galactose and 1% raffinose to induce *pGAL-INT1* expression, were grown overnight again, and were then examined to determine the percentage of cells exhibiting filaments and filament length by using a Nikon Eclipse E800 photomicroscope (Fryer Co., Huntley, Ill.) equipped with differential interference contrast optics. To assay *C. albicans* hyphal growth, cells were grown in yeast-peptone-dextrose medium (YPD) containing adenine, uridine, and 20% fetal calf serum or in RPMI medium (Life Technologies, Rockville, Md.) containing 20% fetal calf serum.

To examine the actin cytoskeleton, induced cultures were stained with rhodamine-phalloidin and examined using epifluorescence microscopy using a modification of the original protocol described by Adams and Pringle (2) as modified by David Amberg (http://genome-www.stanford.edu/group/botlab/protocols/rho_pha_calc.html).

Cells were photographed using a 40×, 0.75-na plan fluor objective. Digital images were collected using a CoolCam liquid-cooled, three-chip color charge-coupled device camera (Cool Camera Company, Decatur, Ga.) and captured to a Pentium II 300 MHz personal computer using Image Pro Plus version 4.0 software (Media Cybernetics, Silver Spring, Md.)

Complementation studies. Strain YJB4686 was transformed with pYES2-CaSLA2 or pYES2, and transformants were selected on SC–plus glucose–lacking leucine medium at 25°C. Transformants were grown overnight, and 10-fold serial dilutions were spotted on SC–plus glucose–lacking leucine (to repress expression of *CaSLA2*) and SC plus galactose–lacking leucine (to induce expression of *CaSLA2*) media and grown at 25 and 37°C.

Generation of *C. albicans sla2* disruptants. The *SLA2* disruption cassette was constructed from pJB1001, a plasmid containing the 2.9-kb *SLA2 Hin*dIII fragment inserted into the *Hin*dIII site of pUC18 digested with *Eco*RV, which cuts between codons 713 and 714 within the *SLA2* open reading frame. *URA*-blaster plasmid pMB7 (21) was digested with *Pvu*II to liberate a 4.1-kb fragment which was gel purified and ligated into *Eco*RV-digested pJB1001 to generate pJB1064.

For gene disruptions, *CaSLA2-hisG*::*URA3*::*hisG-CaSLA2* was released from pJB1064 by digestion with *Pvu*II and was used to transform *C. albicans* strain CAI4 (21) for uracil prototrophy. Insertion of the *URA*-blaster cassette within the *CaSLA2* locus in strains YJB3400 and yCA37 was confirmed by PCR with primers P1 (5′-AGA TAA TGC TCT TGC TGA-3′), P2 (5′-TTC CCA TCG ATA ACA GCA-3′), and P3 (5′-CGA CTT CGA CAG AAC CAT-3′) and Southern analysis (data not shown). Independent heterozygotes were then plated onto SC medium containing 2% glucose, uridine, and 5-fluoroorotic acid (FOA) to select for loss of the *URA3* marker (22). FOA-resistant *CaSLA2*/*Casla2* (YJB3401 and YJB3611) strains were then retransformed with *Pvu*II-digested pJB1064 to disrupt the second *CaSLA2* allele. Homozygous *Casla2*/*Casla2* strains were identified by restriction patterns on Southern blots, including the characteristic loss of the 2.7-kb *Hin*dIII band (data not shown), and identifications were verified by PCR. Two independent homozygous *Casla2*/*Casla2* strains (YJB3402 and YJB3612) were generated to ensure that any phenotype was due to disruption of *CaSLA2* rather than to spurious mutations that can occur during the transformation process.

TABLE 1. Plasmids and yeast strains used

Strain or Plasmid	Relevant features or genotype	Source or reference
Plasmids		
pBM272	*ampR URA3 CEN4 ARS1 pGAL1 pGAL10*	33
pCG01	pBM272 *pGAL10-INT1*	23
pCG110	pCG01 *ura3*::*LEU2*	This study
pCG108	pCG108 *ura3*::*TRP1*	This study
pYES2	*ampR URA3* 2μm ori *pGAL1*	Invitrogen, Inc.
pYES2-*CaSLA2*	pYES2 *pGAL1-CaSLA2*	This study
S. cerevisiae		
Actin charge-to-alanine scanning mutants		
YJB2603	*MAT***a** *ACT1*::*HIS3 his3-Δ200 leu2-3,112 ura3-52 ade4 tub2-201* [*pCG01*]	67
YJB2604	YJB2603 *act1-101*::*HIS3 can1* [*pCG01*]	67
YJB2606	YJB2603 *act1-113*::*HIS3 can1* [*pCG01*]	67
YJB2607	YJB2603 *act1-119*::*HIS3* [*pCG01*]	67
YJB2608	YJB2603 *act1-120*::*HIS3* [*pCG01*]	67
YJB2609	YJB2603 *act1-122*::*HIS3* [*pCG01*]	67
YJB2610	YJB2603 *act1-124*::*HIS3* [*pCG01*]	67
YJB2611	YJB2603 *act1-129*::*HIS3* [*pCG01*]	67
YJB2612	YJB2603 *act1-133*::*HIS3* [*pCG01*]	67
YJB2614	YJB2603 *act1-104*::*HIS3* [*pCG01*]	67
YJB2615	YJB2603 *act1-115*::*HIS3* [*pCG01*]	67
YJB2617	YJB2603 *act1-117*::*HIS3* [*pCG01*]	67
YJB2618	YJB2603 *act1-123*::*HIS3* [*pCG01*]	67
Actin cytoskeleton mutants		
YJB2714	*MAT*α *ABP1 ura3-52 leu2-3* [*pCG01*]	69
YJB2715	*MAT***a** *abp1*::*LEU2 ura3-52 lys2-801 am leu2-3,112* [*pCG01*]	69
YJB3054	*MAT***a** *SAC6 his3 leu2 lys2 ura3* [*pCG01*]	A. Adams
YJB3053	YJB3054 *sac6*::*LEU2* [*pCG01*]	A. Adams
YJB3321	*MAT***a**/α *BNI1*/*BNI1 DFG9*/*DFG9 SRV2*/*SRV2 ura3-52*/*ura3-52* [*pCG01*]	41
YJB3331	*MAT***a**/*MAT*α *bni1-100*/*bni1-100 ura3-52*/*ura3-52 leu2*::*hisG*/*leu2*::*hisG trp1*::*hisG*/*TRP1* [*pCG01*]	53
YJB3329	*MAT***a**/*MAT*α *srv2-100*/*srv2-100 ura3-52*/*ura3-52 leu2*::*hisG*/*leu2*::*hisG trp1*::*hisG*/*TRP1* [*pCG01*]	53
YJB2457	*MAT***a**/*MAT*α *dfg9-100*/*dfg9-100 ura3-52*/*ura3-52 leu2*::*hisG*/*leu2*::*hisG trp1*::*hisG*/*TRP1* [*pCG01*]	53
YJB3057	*MAT*α *RVS167 his3Δ200 leu2-3,112 ura3-52 trp1-1(am) lys2-801(0c)* [*pCG01*]	39
YJB3056	*MAT*α *rvs167*::*TRP1 his3Δ200 leu2-3,112 ura3-52 trp1-1(am) lys2-801(0c)* [*pCG01*]	39
YJB2623	*MAT***a** *VRP1 ura3-52 ade1 leu2-3,112 ile MEL1* [*pCG01*]	A. Hopper
YJB2622	*MAT*α *vrp1*::*LEU2 leu2-3,112 ade1 ura3-52 ile MEL1* [*pCG01*]	72
YJB3156	*MAT***a** *SLA1 SLA2 leu2-3,112 ura3-52* [*pCG110*]	28
YJB3155	*MAT***a** *sla1-Δ1*::*URA3 leu2-3,112 ura3-52* [*pCG110*]	28
YJB3157	*MAT*α *ura3-52 leu2-3,112 sla2Δ*::*URA3* [*pCG110*]	69
YJB2489	*MAT***a** *ura3-52 leu2-Δ2 his3-Δ200 trp1-Δ63 lys2-801*	Mark Longtine
YJB4786	YJB2489 *sla2*::*HIS3*	This study
cdc3 strains		
YJB2757	*MAT*α *ura3-52 leu2-Δ2 his3-Δ200 trp1-Δ63 lys2-801(SLA2)*	9
YJB3857	YJB2757 *sla2*::*HIS3*	This work
YJB5565	*MAT***a** *ura3-52 leu2-Δ2 his3-Δ200 trp1-Δ63 lys2-801 cdc3-6*	This work
YJB5566	*MAT***a** *ura3-52 leu2-Δ2 his3-Δ200 trp1-Δ63 lys2-801 cdc3-6 sla2*::*HIS3*	This work
Sla2/*End4* domain deletion strains		
YJB4553	*MAT***a**/*MAT*α *SLA2*/*SLA2 ura3-52*/*ura3-52 leu2-3,112*/*leu2-3,112 lys2-801*/*lys2-801 his3-Δ200*/*his3-Δ200*/*his3-Δ200 ade2-1*/*ADE2* [*pCG110*]	70
YJB5176	YJB4553 *sla2Δ*::*URA3*/*sla2Δ*::*URA3* [*pCG110*]	70
YJB4550	YJB4553 *sla2Δ768-968*::*URA3*/*sla2Δ768-968*::*URA3* [*pCG110*]	70
YJB4549	YJB4553 *sla2Δ501-968*::*URA3*/*sla2Δ501-968*::*URA3* [*pCG110*]	70
YJB4552	YJB4553 *sla2Δ360-968*::*URA3*/*sla2Δ360-968*::*URA3* [*pCG110*]	70
YJB5163	*MAT***a** *sla2-Δ1*::*URA3 his3-Δ200 leu2-3,112 ura3-52* [*pCG110, pRS313*]	70
YJB5164	YJB5163 [*pCG110, pDD353* (*SLA2*)]	70
YJB5165	YJB5163 [*pCG110, pDD362* (*sla2Δ33-750*)]	70
YJB5166	YJB5163 [*pCG110, pDD364* (*sla2Δ33-501*)]	70
YJB5167	YJB5163 [*pCG110, pDD367* (*sla2Δ33-359*)]	70
YJB5168	YJB5163 [*pCG110, pDD369* (*sla2Δ33-359, 576* stop)]	70
YJB5169	YJB5163 [*pCG110, pDD371* (*sla2Δ360-575*)]	70
YJB3149	*MAT***a** *his3 lys2 ura3 leu2 bar1 end4*::*HIS3 END4*:*TRP1* [*pCG110*]	68
YJB3146	YJB3149 *end4Δ114-284*:*TRP1* [*pCG110*]	68
YJB3154	YJB3149 *end4Δ286-301*:*TRP1* [*pCG110*]	68

Continued on following page

TABLE 1—*Continued*

Strain or Plasmid	Relevant features or genotype	Source or reference
YJB3147	YJB3149 *end4Δ318-373:TRP1* [*pCG110*]	68
YJB3150	YJB3149 *end4Δ376-501:TRP1* [*pCG110*]	68
YJB3151	YJB3149 *end4Δ495-573:TRP1* [*pCG110*]	68
YJB3152	YJB3149 *end4Δ376-573:TRP1* [*pCG110*]	68
YJB3153	YJB3149 *end4Δ376-440:TRP1* [*pCG110*]	68
YJB3148	YJB3149 *end4Δ767-968:TRP1* [*pCG110*]	68
Strains used in complementation studies		
YJB4686	*MAT***a** *lys2 his4 leu2 ura2 bar1 end4 sla2::LEU2 GAL*	H. Reizman
YJB4896	YJB2757 [*pCG108, pYES-CaSLA2*]	This study
YJB4897	YJB3857 [*pCG108, pBM272*]	This study
YJB4897	YJB3857 [*pCG108, pYES2*]	This study
YJB4898	YJB3857 [*pCG108, pYES-SLA2*]	This study
C. albicans strains		
YJB1873 (CAF2)	*ura3::imm434/URA3 CaSLA2/CaSLA2*	21
YJB3018 (CAI4)	*ura3::imm434/ura3::imm434 CaSLA2/CaSLA2*	21
YJB3400	YJB3018 *Casla2::hisG::URA3::hisG/CaSLA2*	This study
YCA37	YJB3018 *Casla2::hisG::URA3::hisG/CaSLA2*	This study
YJB3401	YJB3400 *Casla2::hisG/CaSLA2*	This study
YJB3611	YCA37 *Casla2::hisG/CaSLA2*	This study
YJB3402	YJB3401 *Casla2::hisG/Casla2::hisG::URA3::hisG*	This study
YJB3612	YJB3611 *Casla2::hisG/Casla2::hisG::URA3::hisG*	This study

RESULTS

Actin distribution is highly polarized in *INT1*-expressing cells. During hyphal growth in *C. albicans*, the actin cytoskeleton is also highly polarized to the growing hyphal tip (5). During both bud growth and shmoo formation, as well as during pseudohyphal growth, *S. cerevisiae* cells polarize their cytoskeleton towards zones of growth (18, 69). To monitor actin organization in *S. cerevisiae* cells expressing Int1p, we used rhodamine-phalloidin to visualize actin cables and cortical patches (1). In wild-type cells, actin cortical patches initially localize to the site of incipient bud growth and then to the tip of small buds. Once a critical bud size is reached, the cortical patches are distributed over the entire bud surface, resulting in isotropic growth of a round bud. Prior to cytokinesis, actin patches are concentrated at the mother-bud neck (reviewed in references 11 and 36). In *INT1*-induced filamentous cells, as in other polarized buds (14), the majority of the actin patches were usually at the growing tips of the cells (Fig. 1). We also observed a few actin patches distributed throughout the polarized bud. We did not observe any concentration of actin patches in the region where the mother and bud meet. Thus, expression of Int1p caused a reorganization of the actin cytoskeleton, such that it was often concentrated near the distal tip of the filamentous bud for a much longer period of time than in cells that do not express *INT1*. Actin patches in the *INT1*-expressing cells appeared similar in size to those in cells expressing only vector sequences.

Two subdomains of actin are required for *INT1*-induced filamentous growth. A large series of actin alleles have been generated to study the structure-function relationships of this important cytoskeleton component (11, 55, 67). We tested strains containing 13 different actin alleles for the ability to generate highly polarized buds in response to *INT1* expression (Table 2). Only 2 of these 13 alleles, *act1-124* and *act1-129*, caused a significant reduction in the extent of *INT1*-induced filamentous growth. In both of these strains, the proportion of cells producing polarized buds was significantly reduced with the most dramatic reduction seen with the *act1-129* allele. Furthermore, when *act1-124* or *act1-29* cells that exhibited some polarized growth were observed, the polarized bud was much shorter than polarized buds induced by *INT1* in otherwise wild-type cells (Fig. 2). The *act1-124* allele maps to subdomain 2 of actin while the *act1-129* allele maps to subdomain 3. The *act1-124* and *act1-129* alleles confer temperature sensitivity at 37°C. However, six other temperature-sensitive actin alleles did not have a significant effect on *INT1*-induced filamentous growth. Thus, it cannot be the ability to grow at high temperature, per se, that is required for the formation of polarized buds.

The *act1-124* and *act1-129* alleles also cause a random budding pattern (14). However, the *act1-120* allele, which also causes a randomized budding pattern, did not have a major effect on *INT1*-induced filamentous growth (Table 2). Furthermore, *INT1*-induced filamentous growth was observed in strains carrying *act1* alleles that exhibit either unipolar or bipolar budding patterns (Table 2). Thus, *INT1*-induced filamentous growth occurs independently of any specific bud site selection pattern.

Interestingly, *act1-120* is a temperature-sensitive actin allele that also is required for cell elongation and invasion of the agar during pseudohyphal growth (14). Yet, the *act1-120* mutation did not have an obvious effect on *INT1*-induced filamentous growth (Fig. 2 and Table 2). The *act1-120* allele is of particular interest because, unlike *act1-124* and *act-129*, the temperature-sensitive phenotype of *act1-120* is suppressed by specific alleles of *SAC6*, which encodes the *S. cerevisiae* fimbrin homolog. This suggests that the actin domain that interacts with Sac6p/fimbrin is not critical for *INT1*-induced filamentous growth.

***INT1*-induced filamentous growth requires a subset of actin-interacting proteins.** To determine which actin-interacting pro-

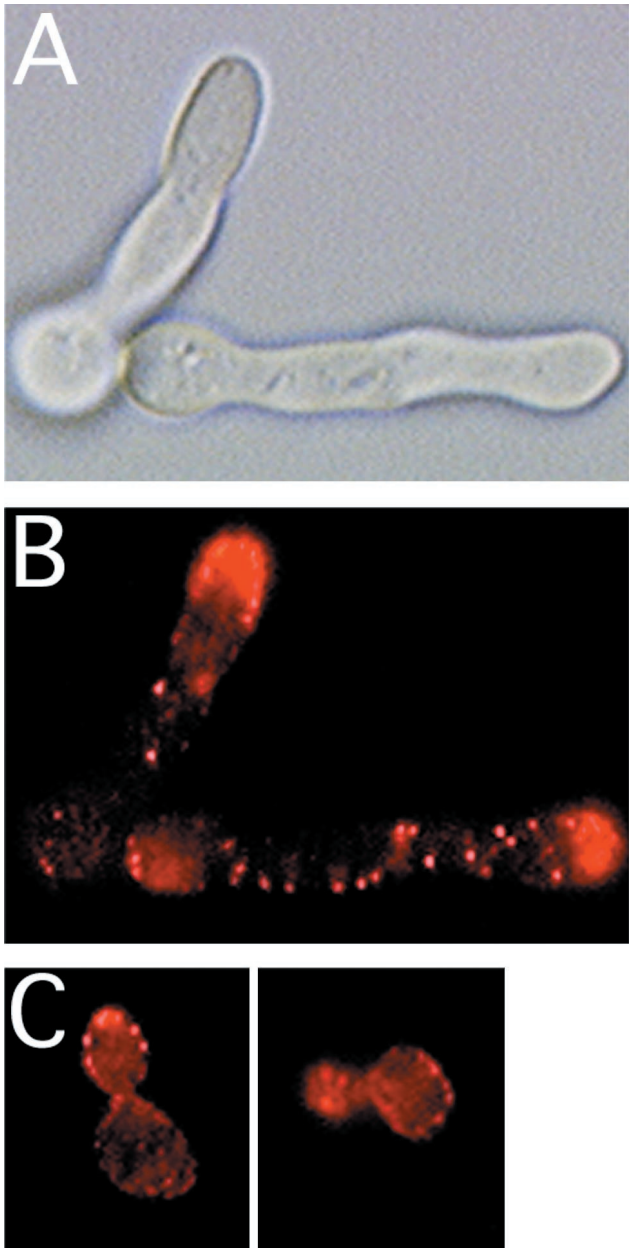

FIG. 1. Actin is highly polarized in *S. cerevisiae* cells expressing *INT1*. DIC images (A) and fluorescence images (B and C) of rhodamine-phalloidin-stained strain YJB2603 cells expressing *INT1* (A and B) after 12 h of growth on galactose. (C) Rhodamine-phalloidin-stained YJB2603 cells were grown on glucose (to repress *INT1* expression).

teins are required for *INT1*-induced filamentous growth, we analyzed the ability of *INT1* to generate highly polarized buds in strains with mutations in several actin-interacting proteins (Fig. 3; Table 3). Several of the mutants studied, including *abp1*::*LEU2* and *sac6*::*LEU2* mutants, had no discernable effect on *INT1*-induced filamentous growth. The observation that *INT1*-induced filamentous growth does not require *SAC6* and that it occurs in the *act1-120* strain is consistent with the idea that fimbrin and the interaction(s) between actin and fimbrin are not important for *INT1*-induced filamentous growth. Interestingly, the *dfg9-100* allele of *PEA2* (Table 3) which disrupts pseudohyphal growth (52) did not affect *INT1*-induced filamentous growth.

Importantly, there were several mutant strains in which *INT1*-induced filamentous growth was significantly reduced. Among this group of strains, the *sla2/end4* strain had a dramatic effect on *INT1*-induced filamentous growth (Fig. 3). *SLA2/END4* encodes a protein involved in membrane cytoskeleton assembly (38), the internalization phase of endocytosis (56, 68) and pseudohyphal growth (69). *SLA2* was isolated, together with *SLA1*, in a screen for mutants synthetically lethal with a disruption allele of *ABP1* (*abp1*::*LEU2*) (28). *SLA2* was also isolated as *END4*, based on its role in endocytosis (60), and as *MOP2*, because it affects the accumulation and/or maintenance of plasma membrane H(+)-ATPase on the cell surface (57). *SLA1* encodes a protein involved in the assembly of the cortical actin cytoskeleton (6, 28). Mutations in *SLA1*, as well as those in *SRV2, VRP1*, and *BNI1*, and to a lesser degree *RVS167*, all reduced the proportion of cells exhibiting *INT1*-induced filamentous growth and in many cases caused a significant reduction in the length of any *INT1*-induced filaments that were formed (Table 3). Thus, several components of the actin cortical patches contribute to efficient *INT1*-induced filamentous growth in *S. cerevisiae*.

Sla2p is specifically required for *INT1* function in *S. cerevisiae*. Because Sla2p is a component of actin cortical patches and because Sla2p is required for pseudohyphal growth (69), we asked if Sla2p is necessary for several types of polarized growth in *S. cerevisiae*. *CDC3* encodes the Cdc3p septin protein found at the mother-bud neck. Loss of septin function causes the formation of cells with highly polarized buds (reviewed in reference 43) that are reminiscent of *INT1*-induced filamentous cells (Fig. 4A). We grew *cdc3-6* and *cdc3-6 sla2* strains and shifted them to 37°C for 12 h to determine if *SLA2* is required for polarized growth. Interestingly, similar elongated buds were observed in both strains (Fig. 4A), indicating that *SLA2* is not required for the generation of filamentous cells per se. Furthermore, this result demonstrates that mutation of *SLA2* does not suppress a *cdc3* mutation. In addition, we asked if Sla2p is required for the polarization of mating projections in response to mating pheromone. Consistent with a previous report (55), *MAT***a** *sla2*Δ strains form mating projections in response to α-factor treatment (Fig. 4B), indicating that they are not defective in the ability to polarize the actin cytoskeleton in response to some stimuli. Interestingly, like *sla2* budding cells (55), the necks of the mating projections appeared to be wider than those in wild-type cells.

We also generated highly polarized cells by expressing high levels of *SWE1*, which encodes the kinase that phosphorylates Clb2/Cdc28 to execute the morphogenesis checkpoint (35), from the *GAL1* promoter on plasmid pSWE1-19 (10). As seen with the *cdc3-6* mutation, cells grown on galactose to induce *SWE1* expression formed highly polarized buds, and the degrees of polarized growth were similar in *SLA2* and *sla2* strains (E. S. Bensen, data not shown). Thus, Sla2p is not required for all forms of polarized growth in *S. cerevisiae* and is not required for *SWE1* to mediate a polarized growth response. Rather, Sla2p is required for certain types of polarized growth, such as filamentous growth, in response to *INT1* expression and pseudohyphal growth in response to nutrient depletion (69),

TABLE 2. Some actin alleles affect *INT1*-induced filamentous growth

Actin allele[a]	Amino acids changed to alanine[b]	Actin subdomain mutated[c]	*INT1*-induced filamentous growth[d]	Filamentous colony growth[e]	Bud site selection pattern[f]	Synthetic lethality with[g]
ACT1			+++	+++	Unipolar	
Temperature sensitivity						
act1-101	363, 364	1	+++			*sla2*Δ
act1-113	210, 211	4	+++	+++	Unipolar	*sac6*Δ
act1-119	116, 117, 118	1	+++			*sac6*Δ
act1-120	99, 100	1	++	−	Random	*sla2*Δ
act1-122	80, 81	2	+++			
act1-124	56, 57	2	+	−	Random	*sla2*Δ, *sac6*Δ
act1-129	177, 179	3	−	−	Random	*sla2*Δ, *sac6*Δ, *abp1*Δ
act1-133	24, 25	1	+++			*sla1*Δ, *sla2*Δ
Pseudo-wild type						
act1-104	315, 316	3	++	+	Unipolar	
act1-115	195, 196	4	+++			*sla2*Δ, *sac6*Δ
act1-117	183, 184	4	+++	++	Bipolar	
act1-123	68, 72	2	+++			

[a] Strains used were YJB2603 to YJB2618, as listed in Table 1.
[b] Phenotypes are as described by Wertman et al. (67).
[c] Domains are as assigned by Botstein et al. (11).
[d] Proportion of cells forming elongated filamentous cells. +++, like wild type; ++, 25 to 75% of wild-type levels; +, 5 to 25% of wild-type levels; −, less than 2% of wild-type levels.
[e] Filamentous growth was described by Cali et al. (14). +++, like wild type; ++, 80 to 100% of colonies had filaments, but filaments were disorganized relative to the wild type; +, 1 to 10% of colonies had filaments; −, no filaments extending beyond the perimeter of the colony.
[f] Bud site selection was determined by Cali et al. (14).
[g] Data were reviewed by Botstein et al. (11).

but Sla2p is not required for polarization of the cytoskeleton during early stages of bud growth or in response to pheromone stimulation during mating (Fig. 4B) (55).

***SWE1* and *SLA2* both contribute independently to *INT1*-induced filamentous growth.** There are a number of mutations in *S. cerevisiae* that generate polarized buds by altering the progression of the mitotic cell cycle. In response to perturbations of the actin cytoskeleton, Swel kinase phosphorylates the Clb2/Cdc28p cyclin-dependent kinase, thereby preventing or delaying the normal transition from polarized growth to isotropic bud growth and delaying nuclear division (35, 37, 49). When stained with DAPI (4′,6′-diamidino-2-phenylindole), *INT1*-induced filamentous cells often have more than one nucleus (C. A. Gale, M. Gerami-Nejad, M. McClellan, M. S. Longtine, and J. Berman, submitted for publication), indicating that the nuclear cell cycle continues, albeit at a reduced rate in some genetic backgrounds. Since Sla2p is a component of actin cortical patches, we determined if *INT1* expression activates the morphogenesis checkpoint by comparing *INT1*-induced filamentous growth in isogenic wild-type and *swe1* null strains (Fig. 5). *INT1*-induced filamentous growth was reduced by 18% in a *swe1*Δ strain relative to growth in the wild-type strain, indicating that Swe1p, and presumably the morphogenesis checkpoint, contributes to, but is not absolutely required for, *INT1*-induced filamentous growth. In isogenic *sla2* strains, *INT1*-induced filamentous growth was reduced by 42% relative to wild-type growth, suggesting that *SLA2* makes an important contribution to, but is also not absolutely required for, this filamentous growth. We then asked if Sla2p is required to mediate *INT1*-induced filamentous growth through the *SWE1*-dependent pathway by comparing filamentous growth in isogenic *sla2, swe1*, or *sla2 swe1* strains. If Sla2p activated *INT1*-induced filamentous growth only through a Swe1-dependent pathway, we would expect to find similar levels of *INT1*-induced filamentous growth in *sla2* and *sla2 swe1* mutant strains. In contrast to this expectation, we found that *sla2 swe1* mutants displayed a much lower degree of *INT1*-induced filamentous growth (97.5% reduction) than did either the *sla2* or *swe1* mutant alone (Fig. 5). Thus, Swe1p and Sla2p do not mediate *INT1*-induced filamentous growth through a single, shared pathway. While these results do not rule out the possibility that Sla2p may trigger some *INT1*-induced filamentous growth by activating the morphogenesis checkpoint, it implies that Sla2p also acts in a Swe1p-independent manner to mediate filamentous growth in cells expressing *INT1*.

The talin-like C terminus of Sla2p is not required for *INT1*-induced filamentous growth. Sla2p is the *S. cerevisiae* protein most closely related to the actin-binding protein talin. Sla2p also shares significant similarity with the human Huntingtin interacting protein 1 (HIP1) and the Hip1-related protein (HIP1R). The C terminus of Sla2p is 45% similar to the C-terminal 197 residues of mouse talin (Fig. 6A) (46), a protein that mediates interactions between integrins at the cell surface and the actin cytoskeleton (30, 34, 54). The C-terminal I/LWEQ motif within the talin-like domain of Sla2p can bind to yeast or vertebrate F-actin in in vitro binding assays, cosediments with F-actin (46), and interacts with actin in two-hybrid assays (70). Because *INT1* encodes a protein with limited similarity to vertebrate α_X and α_M integrins (23), the contribution of Sla2p to *INT1*-induced filamentous growth raised the intriguing possibility that the vertebrate paradigm of integrin-talin-actin interaction may hold for Int1p, Sla2p, and actin in budding yeasts. To test this possibility, we analyzed the domains of Sla2p that are required for *INT1*-induced filamentous

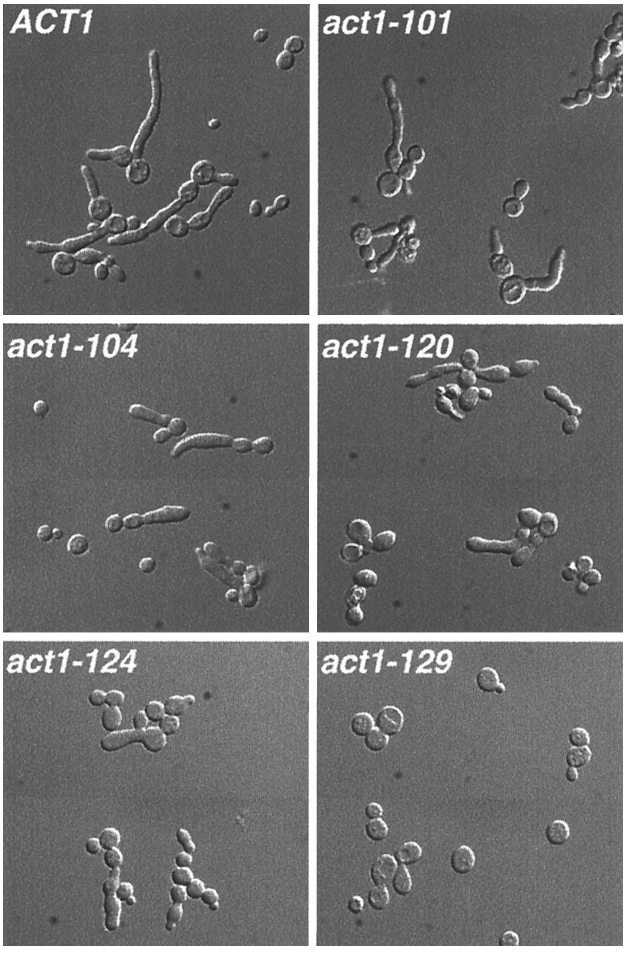

FIG. 2. *INT1*-induced filamentous growth is reduced by specific actin mutations. Shown are DIC micrographs of strains carrying the indicated *ACT1* alleles (*ACT1*, YJB2603; *act1-101*, YJB2604; *act1-104*, YJB2614; *act1-124*, YJB2610; *act1-129*, YJB2611; *act1-120*, YJB2608) and expressing *INT1* after growth on galactose for 16 h. Strains are listed in Tables 1 and 2.

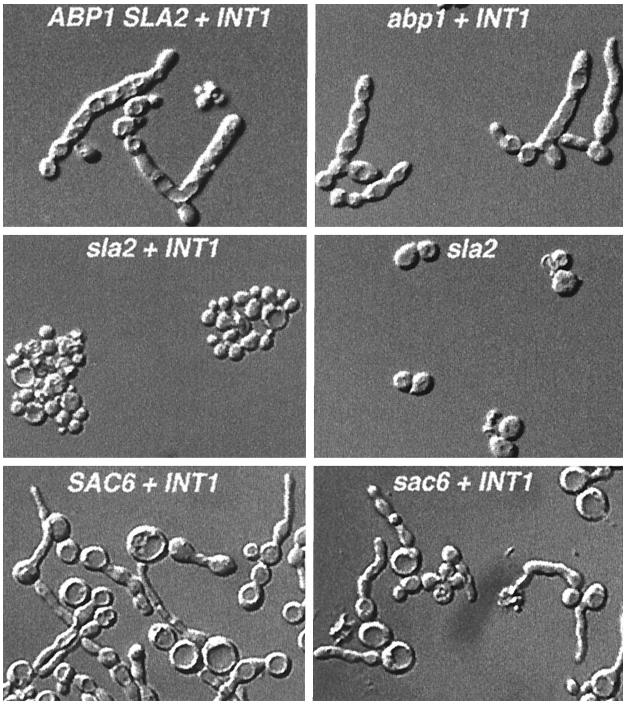

FIG. 3. *INT1*-induced filamentous growth requires *SLA2*, but not *ABP1* or *SAC6*. Shown are DIC micrographs of isogenic wild-type and mutant strains with the relevant genotype indicated. Strains are listed in Tables 1 and 3.

growth using deletion alleles kindly provided by Wesp and coworkers (68) and by Yang and coworkers (70).

Sla2p includes several distinct domains, including a 197-amino-acid (aa) coiled-coil domain required for homodimerization (70) and for an endocytic function that is redundant with the function(s) of Abp1p and Srv2p (68). A small region near the N terminus is required for endocytosis, growth at high temperature, and actin organization (Fig. 6A). Several conclusions can be reached from the analysis of *INT1*-induced filamentous growth in strains carrying deletion alleles of *SLA2* (Fig. 6B). First, the same N-terminal domain that was required for other *SLA2* functions was also an important contributor to *INT1*-induced filamentous growth (Fig. 6B, constructs Δ114-284 and Δ33-359). In addition, deletion of this N-terminal region together with deletion of the coiled-coil domain or the talin-like C terminus caused an almost complete loss of *INT1*-induced filamentous growth (Fig. 6B, Δ33-501 and Δ33-359+Δ576stop). Deletion of both the coil and the talin-like domains reduced *INT1*-induced filamentous growth to less than 50% of the wild-type level, but did not eliminate it completely (Fig. 6B, Δ360-968). Finally, the C-terminal talin-like domain alone was not necessary for *INT1*-induced filamentous growth (Fig. 6B, Δ767-968 and Δ768-968). The latter result is consistent with the previous work of others (68, 70) that indicated that the Sla2p talin-like domain is not required for any known Sla2p/End4p function, although the I/LWEQ boxes within the talin-like domain of Sla2p bind actin in vitro (46). While the Sla2p talin-like domain can interact with actin in vitro, it has been proposed that each Sla2p molecule may be involved in multiple protein-protein interactions with another molecule of Sla2p as well as with other cortical patch proteins (68, 71). Our results are consistent with the idea that multiple interactions with different actin patch components are partially redundant because deletion of any one of them is not sufficient to completely eliminate Sla2p function (68, 71). Thus, while the talin-like domain of Sla2p is not required for *INT1*-induced filamentous growth when other domains are intact, we cannot rule out the possibility that interaction(s) with the actin cortical patches are required for Sla2p to mediate *INT1*-induced filamentous growth. In this context, it is interesting that we detected two classes of cortical patch proteins: those that are required for optimal *INT1*-induced filamentous growth (e.g., Sla1p, Sla2p, Srv2p, and Rvs167p) and those that do not appear to contribute to *INT1*-induced filamentous growth (e.g., Abp1p and Sac6p).

***C. albicans* SLA2 can functionally complement an *S. cerevisiae sla2*Δ mutant.** *C. albicans* Sla2p (CaSla2p) is very similar to *S. cerevisiae* Sla2p (ScSla2p) across the entire length of the protein, including the essential N-terminal region, the coiled-coil domain (aa 376 to 573 in *S. cerevisiae*, aa 350 to 600 in *C.*

TABLE 3. Some actin cortical patch mutations affect *INT1*-induced filamentous growth

Strain name	Relevant genotype[a]	No. of *INT1*-induced filaments[b]	Filament shape[c]
YJB2714	Wild type	+++	
YJB2715	*abp1*Δ	+++	wt
YJB3054	Wild type	+++	
YJB3053	*sac6*Δ	+++	
YJB3321	Wild type	+++	
YJB2520	pea2/dfg9-100	+++	wt
YJB3329	*srv2-100*	+	sh
YJB3331	*bni1-100*	+	sh, shm
YJB2623	Wild type	+++	
YJB2622	*vrp1*Δ	+	sh
YJB3057	Wild type	+++	
YJB3056	*rvs167*Δ	++	sh
YJB3156	wild type	++++	
YJB3155	*sla1*-Δ*1*	+	sh
YJB3156	Wild type	+++	
YJB3157	*sla2*Δ	+	sh

[a] Complete genotypes of the strains are listed in Table 1.
[b] Relative proportion of cells exhibiting *INT1*-induced filaments. In each case, the strain was compared with the appropriate isogenic or congenic wild-type strain listed in Table 1. +++, similar to wild type; ++, ~25 to 75% of wild-type levels; +, less than 25% of wild-type levels of filament formation.
[c] Appearance of filaments relative to how they appeared in the isogenic or congenic wild-type strains. wt, filaments were indistinguishable from wild-type filaments; sh, average filament length was shorter than in the relevant wild-type strain; shm, polarized cells appeared similar to mating projections (shmoos).

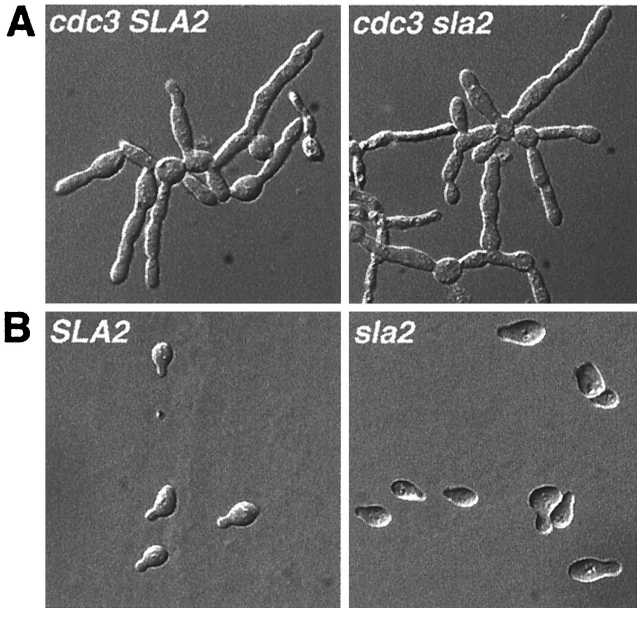

FIG. 4. *SLA2* is not required for all types of polarized growth. (A) DIC micrographs of strains carrying the *cdc3-6 allele*, which results in polarized growth in the presence (YJB5565; left) or absence (YJB5566; right) of *SLA2*. (B) DIC micrographs of *MAT***a** wild-type (YJB2489) and *sla2*Δ (YJB4786) strains exposed to 300 μg of α-factor/ml.

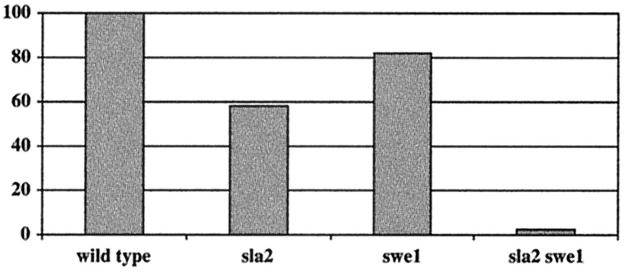

FIG. 5. *SLA2* and *SWE1* contribute independently to *INT1*-induced filamentous growth. The percentage of *INT1*-induced filaments was determined by spreading cells onto plates containing 2% galactose and counting the cells producing filaments and the total number of cells on the plate 18 h after plating. Two isolates transformed with pGAL-*INT1* were used for each experiment, and a minimum of 200 cells was counted for each strain. The wild-type strain produced 95% filamentous cells under these conditions.

albicans), and the talin-like domain (Fig. 6A). The C terminus of CaSla2p is even more similar to mouse talin (40.6% identity, 52.4% similarity) than ScSla2 is similar to mouse talin (37.6% identity, 47.9% similarity). ScSla2p and CaSla2p are also related to Sla2p's of other yeasts (50), talin proteins from *Dictyostelium discoideum* and *Caenorhabditis elegans*, human Hip1p, and other proteins that contain the highly conserved I/LWEQ boxes within the C termini (45).

To ask if CaSla2p executes the same functions as ScSla2p, we expressed *CaSLA2* in an *S. cerevisiae sla2* deletion strain and monitored growth at 37°C as well as the ability to form filamentous cells in response to *INT1* expression. The *sla2* strain expressing only vector sequences (YJB4686 plus pYES) was unable to grow at 37°C, while the same strain expressing pYES2-*CaSLA2* (YJB4686 plus pYES-CaSLA2) was able to grow at 37°C (data not shown). In addition, the extents of *INT1*-induced filamentous growth (both the percentage of cells forming filaments and filament length) were similar in the *Scsla2*Δ strain expressing *CaSLA2* (YJB4898) and an isogenic *ScSLA2* strain (YJB4896) expressing *CaSLA2* (Table 4). It should be noted that the *CaSLA2* coding sequence includes one CUG codon (at aa 161), which encodes serine in *C. albicans* and leucine in all other organisms, and which does not appear to have an essential role in Sla2p function. Thus, ScSla2p and CaSla2p appear to have similar functions in *S. cerevisiae*, at least for growth at high temperature and in response to *INT1* expression.

***CaSLA2* is required for filamentous growth in *C. albicans*.** *C. albicans* undergoes a more complex set of morphogenetic responses to the environment than does *S. cerevisiae* and we wanted to determine if CaSla2p is required for these responses. We sequentially generated disruption alleles of both copies of CaSla2p by insertion of the URA-blaster cassette (21) into the *Eco*RV site between codons 713 and 714 of *CaSLA2*, generating a protein lacking 351 C-terminal amino acids, including the 197-aa talin-like domain of the protein and all of the I/LWEQ boxes found between aa 865 and 1055. Several independent *Casla2*/*CaSLA2* heterozygous strains were isolated and used to generate independent *Casla2*/*Casla2* homozygous strains. Disruption of the gene was detected by PCR and confirmed with Southern blotting (data not shown).

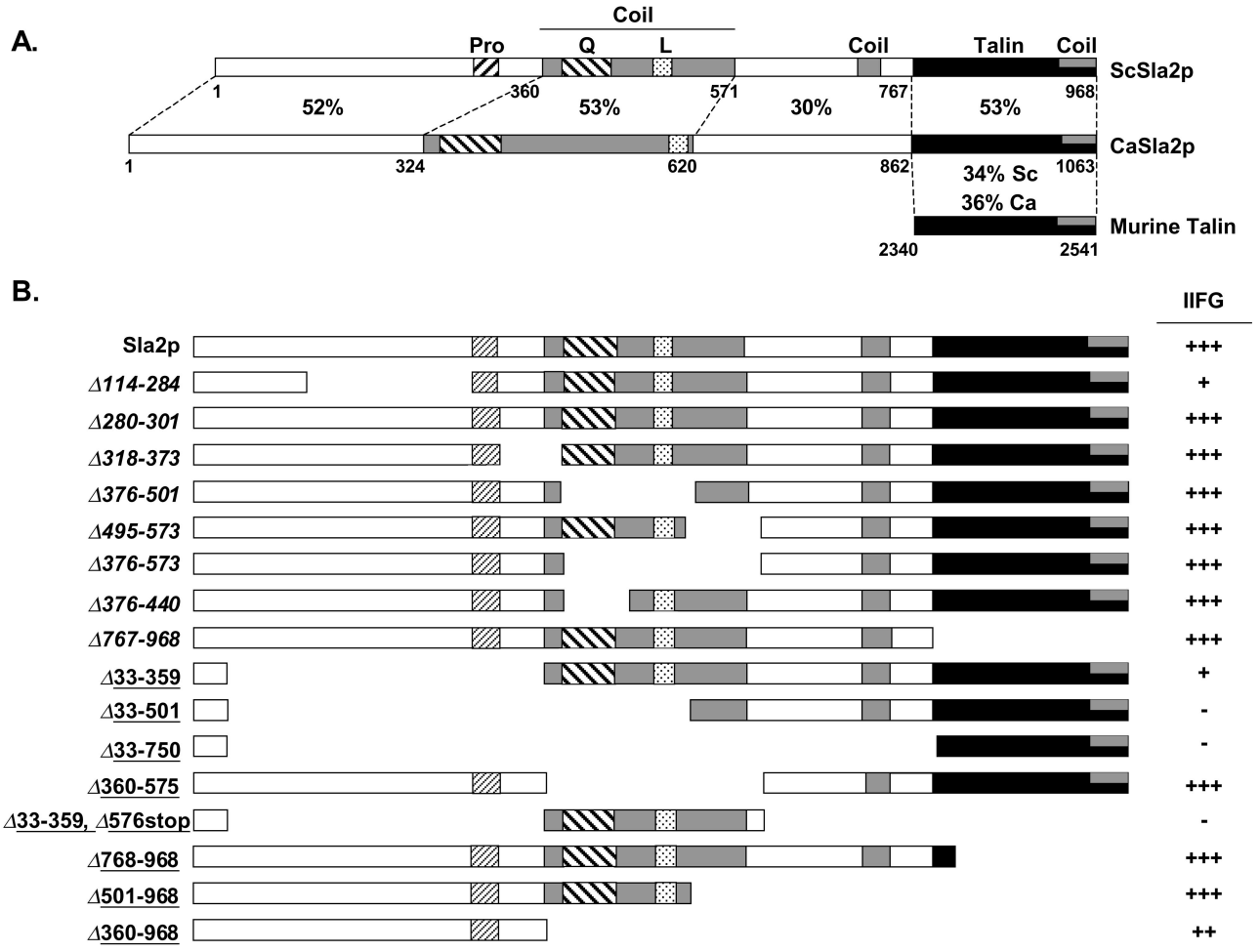

FIG. 6. *S. cerevisiae SLA2* and *C. albicans SLA2* have a talin-like domain which is not required for Sla2p function in *S. cerevisiae*. (A) Comparison of ScSla2p, CaSla2p, and mouse talin domains. Amino acid positions of the domains illustrated are noted below the genes. Percent identity between domains of the proteins is indicated. Pro, proline-rich region; Q and L, glutamine- and leucine-rich regions within the long coil domain. Only the talin domain of murine talin has similarity to the Sla2 proteins. (B) Deletion analysis of *ScSLA2* domains required for *INT1*-induced filamentous growth. Illustration of deletion alleles obtained from Reizman and colleagues (not underscored) and Drubin and colleagues (underscored). IIFG, *INT1*-induced filamentous growth determined using the scoring system described in Table 2.

Two independent strains, YJB3612 and YJB3402, with disruptions only within both *CaSLA2* alleles were chosen for continued study.

The growth and morphology of the heterozygous and homozygous strains were analyzed on different media that induce hyphal growth. On milk-Tween agar, wild-type strains produced lush filamentous growth emanating from the colony (Fig. 7A, left), as did both heterozygous *CaSLA2*/*Casla2* strains (Fig. 7A, middle). In contrast, both of the homozygous *Casla2*/*Casla2* disruption strains formed only smooth colonies (Fig. 7A, right). We also analyzed the effect of disruption of *CaSLA2* on the morphology of individual cells grown in RPMI medium containing 20% fetal calf serum at 37°C. These conditions induced hyphal growth of wild-type cells (Fig. 7B, left panel), and hyphae were also evident in both heterozygous *CaSLA2*/*Casla2* strains (Fig. 7B, middle panel). In contrast, the homozygous *Casla2*/*Casla2* strains failed to form any true hyphae (cells with parallel side walls and perpendicular septa; Fig. 7B, right).

TABLE 4. *C. albicans SLA2* can complement *S. cerevisiae sla2*Δ for *INT1*-induced filamentous growth

Strain name	Relevant genotype	% *INT1*-induced filamentous growth[a]
YJB4896	*SLA2 CaSLA2*	100
YJB4897	*sla2*Δ	47
YJB4898	*sla2*Δ *CaSLA2*	97

[a] Data were normalized relative to *INT1*-induced filamentous growth in YJB4896.

Immunoblot analysis of *C. albicans Casla2*/*Casla2* strains revealed that disruption at the *Eco*RV site eliminated a band with an apparent molecular mass of ~120 kDa and no new bands appeared in the gel (M. McClellan, unpublished data). There were no bands that appeared or displayed an obvious increase in intensity near the mobility expected (~82 kDa) if the predicted truncation product was stable. Thus, the disruption of CaSla2p appears to have generated an unstable protein

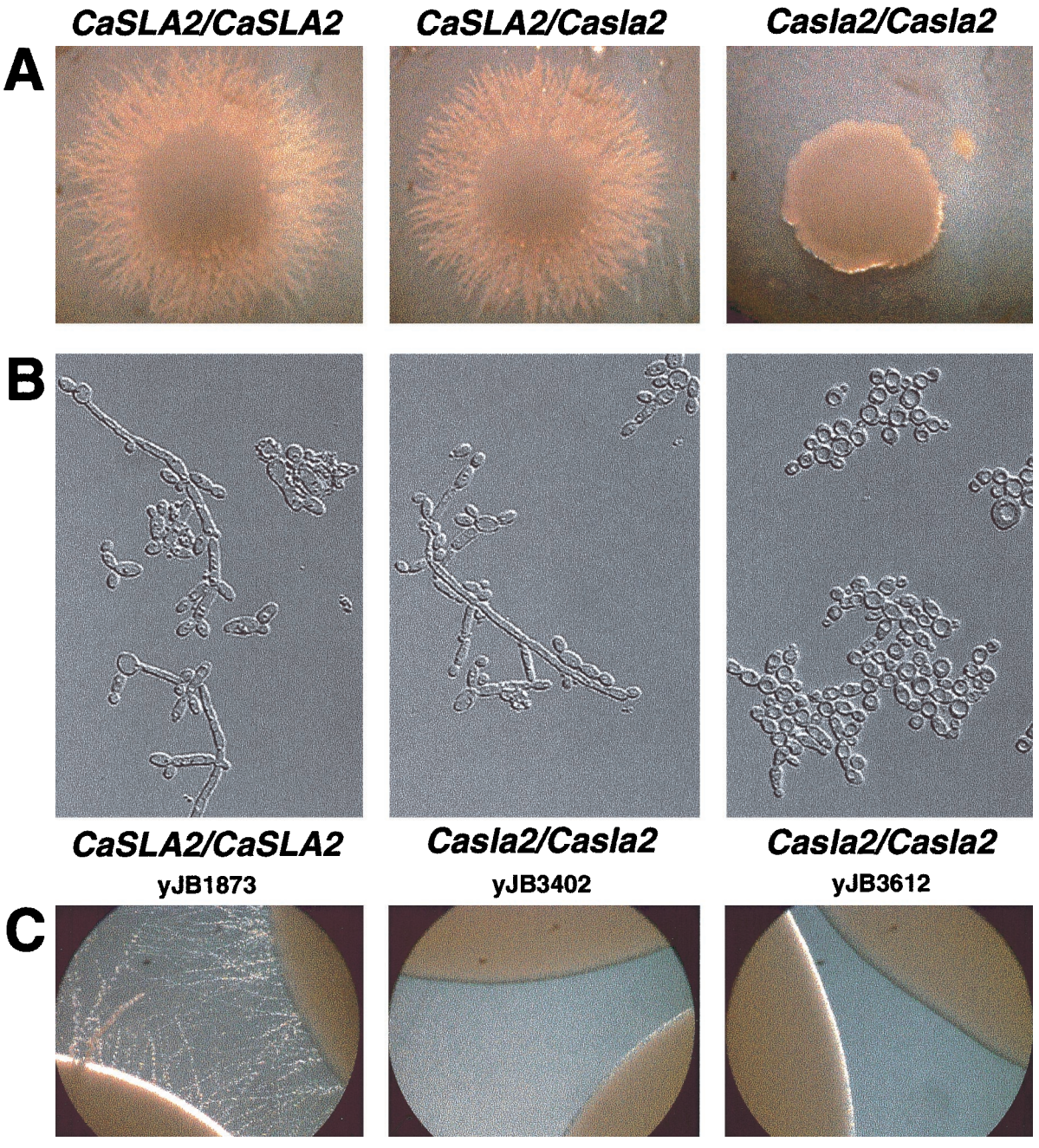

FIG. 7. *CaSLA2* is required for filamentous growth under several different hypha-inducing conditions. *CaSLA2*/*CaSLA2* (YJB1873), *CaSLA2*/*Casla2* (YJB3400), and *Casla2*/*Casla2* (YJB3402) were grown on milk-Tween agar for 5 days (A), in RPMI medium–20% serum for 16 h at 37°C (B), or on YPD agar medium for 13 days (C). Similar results were observed with independently isolated yCA37 and YJB3612 heterozyogous and homozygous *Casla2* strains. (C) Parental and two independent *Casla2*/*Casla2* homozygote strains as indicated were photographed after 13 days of growth on YPAD at room temperature.

and the phenotypes of the *Casla2*/*Casla2* strains are likely to be similar to the phenotypes of *Casla2* null strains.

Many strains (including *cph1*/*cph1 efg1*/*efg1* and *ras1*/*ras1* strains) that are generally unable to form hyphae under strong induction conditions, such as serum at 37°C, still form filaments under microaerophilic or embedded agar conditions or incubation on YPD at room temperature (20, 65). This supports the currently favored model, which posits that different environmental stimuli trigger separate signal transduction pathways that converge on a common group of targets required for hyphal growth (19). We asked if *Casla2*/*Casla2* strains would form filaments under these types of conditions as well. Even after 2 weeks on YPD, no filaments extended from colonies of either of the *Casla2*/*Casla2* strains (Fig. 7C, middle and right). This result suggests that, in *C. albicans*, Sla2p is important for hyphal growth in response to both mild (e.g.,

milk-Tween agar and long incubation on YPD) and potent (serum at 37°C) environmental stimuli. In fact, in *C. albicans*, CaSla2p appears to be required for the formation of filaments under all hyphal induction conditions tested.

DISCUSSION

Contribution of the actin cytoskeleton to *INT1*-induced filamentous growth in *S. cerevisiae*. We have used the heterologous expression of a *C. albicans* gene, *INT1*, in *S. cerevisiae* to study the contribution of the actin cytoskeleton to polarized growth. We found that actin patches appear normal in size and are often concentrated at the tips of the growing filaments. Furthermore, a small subset of actin mutations affects *INT1*-induced filamentous growth. Both actin alleles that disrupt *INT1*-induced filamentous growth confer temperature sensitivity at 37°C and can disrupt pseudohyphal growth when wild-type actin is also present (14). The *act1-129* allele alters residues 177 and 179 in subdomain 3 of actin. These amino acids are predicted to stabilize actin-actin contacts, and mutations in them cause defects in actin-actin interactions in two-hybrid assays and can disrupt actin filament assembly in a dominant manner (3, 26, 44). These results also highlight differences in the genetic requirements for pseudohyphal growth and *INT1*-induced filamentous growth. For example, the *act1-120* mutation, which perturbs interactions of actin with fimbrin (Sac6p) (3, 4, 27, 29) and is required for pseudohyphal growth (14), had very little effect on *INT1*-induced filamentous growth (Fig. 2). Consistent with this, *sac6*Δ strains that perturb pseudohyphal growth (14) did not perturb *INT1*-induced filamentous growth (Table 3). This result implies that interactions between actin and fimbrin are not important for *INT1*-induced filamentous growth.

In addition, we found that a subset of genes encoding cortical patch constituents is required for *INT1*-induced filamentous growth. These include *SLA1, SLA2*, and *SRV2*. In contrast, Abp1p and Sac6p, which colocalize to the cortical actin cytoskeleton together with Sla1p, Sla2p, and Srv2p (39), are not required for *INT1*-induced filamentous growth. Thus, *INT1*-induced filamentous growth is affected to different degrees by mutations in different proteins associated with the yeast cortical actin patches.

Because of its role in both pseudohyphal growth and *INT1*-induced filamentous growth, we asked if Sla2p is required for the execution of polarized bud growth under all circumstances. Interestingly, we found that *SLA2* is not required for the formation of highly polarized buds in the absence of the Cdc3p septin or in the presence of excess Swe1p (35) and that Sla2p is not required for bud emergence or shmoo formation. Thus, in *S. cerevisiae*, Sla2p has a specific, rather than a general, role in executing polarized growth in response to specific signals.

The N-terminal domains of ScSla2p are essential for *INT1* function. Initially, we were intrigued by the relationship between Int1p, a protein with limited similarity to vertebrate integrins, and Sla2p, the *S. cerevisiae* protein most similar to vertebrate talins, because it raised the possibility that Sla2p might mediate interactions between Int1p and the actin cytoskeleton. The ScSla2p C-terminal talin-like domain binds actin in vitro and in two-hybrid assays (46, 70). However, by analyzing several sets of *sla2* deletion strains for the ability to support Int1p-induced filamentation, we found that the talin-like C terminus of Sla2p, which is dispensable for all known Sla2p functions also, is not required for *INT1*-induced filamentous growth. Despite this finding, deletion of the talin-like domain enhanced the defects in *INT1*-induced filamentous growth seen when either the coiled-coil domain or the N-terminal essential domain were deleted (Fig. 6B). Thus, the talin-like domain may contribute to Sla2p function through actin interactions that are redundant with other interactions between different Sla2p domains and several different components of the cortical actin cytoskeleton (68, 70). Our data are consistent with the idea that the N-terminal domain of Sla2p is most important for function, that the central coil makes an important contribution, likely by facilitating the formation of homodimers (70), and that the talin-like domain makes only a minimal contribution to Sla2p function.

Perhaps Int1p, like Sla2p, interacts with Sla2p and/or other actin cytoskeleton components through more than one domain. For example, it is tempting to speculate that the coil domain of Sla2p and the predicted coiled-coil domains of Int1p (aa 347 to 363, 465 to 479, and 1512 to 1525) may interact. While we cannot rule out this possibility, two-hybrid experiments and several attempts at coprecipitation of Sla2p with Int1p failed to reveal strong evidence for direct, physical interactions between Sla2p and Int1p domains (E. Bensen and M. McClellan, unpublished results). Thus, we also must consider the alternative hypothesis that Int1p may stimulate filamentous growth in *S. cerevisiae* through indirect interactions with Sla2p. Given the large number of genetic and physical interactions between Sla2p and other cytoskeleton proteins, such as Pfy1p, Rvs167p, Sac6p, Abp1p, Ark1p, and actin (15, 27, 68–70), there are many proteins that might mediate an interaction between Int1p and Sla2p.

Role of *SWE1* in *INT1*-induced filamentous growth. When the actin cytoskeleton is perturbed, for example, by cold shock or by treatment with Latrunculin A, the morphogenesis checkpoint is activated by the Swe1p kinase (49). Swe1p phosphorylates Clb2/Cdc28p, thereby preventing the switch to isotropic growth that normally occurs early in the cell cycle (64). Activation of the morphogenesis checkpoint causes cells to remain in the polarized growth state: mutations that trigger this checkpoint often result in highly polarized cells that resemble cells expressing *INT1*. Mutations that affect the septin ring also cause polarized growth in an Swe1-dependent manner (7, 48). Interestingly, the C terminus of *Int1p* has homology to *S. cerevisiae* Bud4p, which is localized to the septin rings at the mother-bud neck. In *S. cerevisiae*, Int1p colocalizes with septins (Gale et al., submitted), suggesting that *INT1*-induced filamentous growth might be dependent upon *SWE1*. While *SWE1* contributes to *INT1*-induced filamentous growth, it is not absolutely required for it (Fig. 5 and 8). While our epistasis analysis indicates that Sla2p and Swe1p clearly make independent contributions to *INT1*-induced filamentous growth, we cannot rule out the possibility that Sla2p also mediates filamentous growth through a Swe1-dependent mechanism (Fig. 8, dashed arrow). Furthermore, there is a small amount of residual filamentous growth that occurs in *sla2 swe1* cells expressing *INT1*, suggesting that there is a third, as-yet-uncharacterized mechanism that mediates filamentous growth in *S. cerevisiae* cells expressing *INT1* (Fig. 8, question mark).

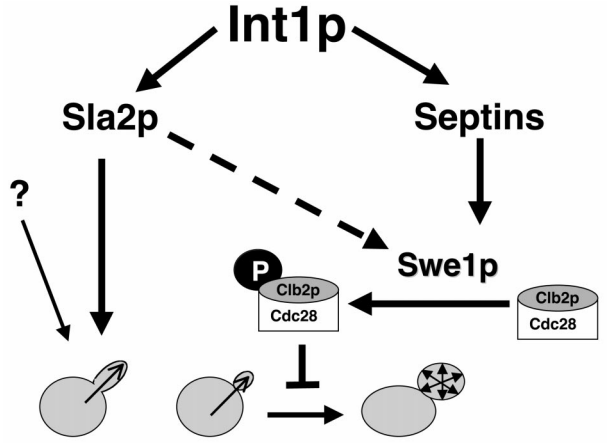

FIG. 8. Int1p triggers filamentous growth through at least two pathways. Sla2p and Swe1p contribute independently to polarized growth in *S. cerevisiae* cells expressing *INT1*. Swe1p may also trigger the morphogenesis checkpoint via Swe1p.

***CaSLA2* is required for hyphal growth under potent inducing conditions.** ScSla2p and CaSla2p have related structures, as revealed by the conservation of sequence motifs within the domains identified as important for Sla2p function in *S. cerevisiae* (50). Furthermore, expression of *CaSLA2* in an *S. cerevisiae sla2*Δ strain restored growth at high temperature and restored *INT1*-induced filamentous growth, indicating functional similarity for the N-terminal domains of both proteins. Yet, the *Casla2* disruption allele we used disrupted the gene ~160 codons upstream of the Sla2p talin-like domain. Western analysis of proteins expressed in these mutants, using anti-ScSla2p antibodies (kindly provided by Drubin and coworkers), suggested that the C-terminally truncated protein, which lacks the talin domain, is unstable in these strains. Thus, the phenotypes observed in the *Casla2*/*Casla2* strains most likely are due to loss of Sla2p function. This is different from what was seen in *S. cerevisiae* cells, where Sla2p that lacks the talin domain remains stable and retains most Sla2p functions.

In *C. albicans*, many different genes are required for hyphal growth in response to environmental stimuli, such as serum, or in response to nutrient deprivation. However, many of the genes that are required for filamentous growth on milk-Tween or Spider agar (e.g., *CPH1* and *INT1*) are dispensable for filamentous growth at 37°C in serum (22, 40). Furthermore, genes such as *EFG1* and *RAS1*, which are required for filamentous growth in response to serum, are not required for filamentous growth under other conditions (20, 65). Even triple-mutation strains lacking *TUP1, EFG1*, and *CPH1* exhibit filamentous growth under some conditions (12, 61), suggesting that there are additional, as-yet-uncharacterized filamentous growth pathways in *C. albicans* (12). This is thought to be due to the activation of multiple independent filamentous growth signals under different sets of hypha-inducing conditions. In contrast to this, *CaSLA2* appears to be required for hyphal growth in response to both nutrient deprivation or serum induction, suggesting that Sla2p may be absolutely required for hyphal growth in *C. albicans*.

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