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Isoform-Specific Complementation of the Yeast *sac6* Null Mutation by Human Fimbrin

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The actin cytoskeleton is a fundamental component of eukaryotic cells, with both structural and motile roles. Actin and many of the actin-binding proteins found in different cell types are highly conserved, showing considerable similarity in both primary structure and biochemical properties. To make detailed comparisons between homologous proteins, it is necessary to know whether the various proteins are functionally, as well as structurally, conserved. Fimbrin is an example of a cytoskeletal component that, as shown by sequence determinations and biochemical characterizations, is conserved between organisms as diverse as *Saccharomyces cerevisiae* and humans. In this study, we examined whether the human homolog can substitute for the yeast protein in vivo. We report here that two isoforms of human fimbrin, also referred to as T- and L-plastin, can both substitute in vivo for yeast fimbrin, also known as Sac6p, whereas a third isoform, I-fimbrin (or I-plastin), cannot. We demonstrate that the human T- and L-fimbrins, in addition to complementing the temperature-sensitive growth defect of the *sac6* null mutant, restore both normal cytoskeletal organization and cell shape to the mutant cells. In addition, we show that human T- and L-fimbrins can complement a sporulation defect caused by the *sac6* null mutation. These findings indicate that there is a high degree of functional conservation in the cytoskeleton, even between organisms as diverse as *S. cerevisiae* and humans.

Fimbrin is an actin filament-bundling protein that is present in organisms from *Saccharomyces cerevisiae* to humans (3, 6, 8, 12, 18). It was first identified in chicken microvilli (7, 12, 20) but was later shown by immunofluorescence microscopy to also be present in nonintestinal cells, largely associated with the membrane at adhesion plaques and the leading edge of lamellipodia (7).

Human fimbrin, also referred to as plastin, was first identified as an extra spot that appeared on two-dimensional gels when cells were transformed (13). More recently, it has become clear that there are at least three isoforms in humans: I-fimbrin, which is expressed in cells that assemble a brush border; L-fimbrin, which is found at the adhesion plaques of leukocytes and transformed solid tissues; and T-fimbrin, which is seen in adhesion plaques of cells in solid tissues (references 16, 18, and 25 and references cited therein). The three proteins are highly conserved in protein sequence, with at least 70% identity to each other and to chicken fimbrin (16, 18). Sequence analysis of these vertebrate fimbrins indicates they all contain an EF-hand calcium-binding domain followed by a pair of α-actinin-like actin-binding domains (8, 18), and biochemical studies have shown that, to various degrees, calcium affects the interaction of these proteins with actin (6, 12, 18, 22).

Yeast fimbrin, also known as Sac6p (3), was previously identified both genetically, through dominant suppression of a temperature-sensitive actin mutation (1), as well as biochemically, on yeast actin filament affinity columns (9). This protein colocalizes with actin in yeast cells (9), bundles yeast actin filaments in vitro (3, 14) and has 36 to 43% identity to human and chicken fimbrins (reference 3 and unpublished data). Sac6p has been shown to be essential for cellular morphogenesis in vivo, as *sac6* null mutant cells, although viable at 23°C, are defective in organization of the cytoskeleton and the generation of normal cell shape (3). The *sac6* null mutant cells fail to grow at 37°C (3). In addition, they are defective in endocytosis (15).

As yeast and vertebrate fimbrins are similar in both primary structure and biochemical activities, we sought to determine whether the yeast and human proteins are functionally conserved and in particular whether they are interchangeable in vivo. This paper reports the finding that human T- and L-fimbrins, but not human I-fimbrin or chicken intestinal fimbrin, can complement the *sac6* null defect in *S. cerevisiae.*

MATERIALS AND METHODS

Yeast strains and methods. Yeast strains used in this study are listed in Table 1. Rich medium (YEP) for nonselective growth and synthetic medium for selective growth of yeast strains, containing either 2% glucose or 2% galactose, have been described previously (26). Yeast transformation was done by the lithium acetate method (11), and sporulation, tetrad dissection, and scoring of markers were done by standard methods (26).

Plasmids. Human T- and L-fimbrin coding sequences (16, 17) were placed under the control of the yeast *GAL1* promoter, using PCR to amplify sequences from the start codon to the stop codon of T-fimbrin (1,893 bp) or L-fimbrin (1,884 bp). In each case, upstream primers contained AAAA just 5′ of the ATG and the adjacent coding sequence, as well as sequences for *Sma*I and *Bam*HI just 5′ of this AAAA (i.e., 5′ CCCGGGGGATCCAAAAATGGATGAGATGGCT ACC 3′ for T-fimbrin and 5′ CCCGGGGGATCCAAAAATGGCCAGAGG ATCAGTGTCC 3′ for L-fimbrin). Downstream primers contained coding sequences and then sequences for *Xba*I and *Sma*I just 3′ of the stop codons (i.e., 3′ CCCTTACTTCTCTCACATTAGATCTGGGCCC 5′ for T-fimbrin and 3′ CCTTACTTCTCCCACACTAGATCTGGGCCC 5′ for L-fimbrin). PCR-generated fragments were digested with *Bam*HI and *Xba*I and inserted into the *Bam*HI-*Xba*I sites of plasmids pTS161 and pTS162 (27). These plasmids are derived from YCp50 (19) and contain the *URA3* gene, *CEN4*, *ARS1*, and the yeast *GAL1/10* promoter (see reference 19 and references cited therein) adjacent to the termination sequence from either the yeast *ACT1* (pTS161) or *ARF1* (pTS162) gene, with *Bam*HI-*Xba*I sites between the *GAL1* promoter and the

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TABLE 1. Yeast strains used in this study[a]

Strain	Genotype	Plasmid (fimbrin)
AAY1047	*MAT***a** *sac6*::*LEU2 his3 leu2 lys2 ura3*	None
AAY1048	*MAT***a** *his3 leu2 lys2 ura3*	None
AAY1069	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 sac6*::*LEU2*/+	None
AAY1415	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 SAC6*$^+$/*SAC6*$^+$	pTS161 (none)
AAY1416	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 sac6*::*LEU2*/*sac6*::*LEU2*	pTS161 (none)
AAY1417	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 SAC6*$^+$/*SAC6*$^+$	pTS162 (none)
AAY1418	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 sac6*::*LEU2*/*sac6*::*LEU2*	pTS162 (none)
AAY1419	*MAT*a/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 SAC6*$^+$/*SAC6*$^+$	AAB184 (L)
AAY1420	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 sac6*::*LEU2*/*sac6*::*LEU2*	AAB184 (L)
AAY1421	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 sac6*::*LEU2*/*sac6*::*LEU2*	AAB186 (L)
AAY1422	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 SAC6*$^+$/*SAC6*$^+$	AAB186 (L)
AAY1423	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 sac6*::*LEU2*/*sac6*::*LEU2*	AAB188 (T)
AAY1424	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 SAC6*$^+$/*SAC6*$^+$	AAB188 (T)
AAY1425	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 sac6*::*LEU2*/*sac6*::*LEU2*	AAB190 (T)
AAY1426	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 SAC6*$^+$/*SAC6*$^+$	AAB190 (T)
AAY1429	*MAT***a** *his3 leu2 lys2 ura3 SAC6*$^+$	pTS161 (none)
AAY1430	*MAT***a** *his3 leu2 lys2 ura3 sac6*::*LEU2*	pTS161 (none)
AAY1437	*MAT*α *his3 leu2 lys2 ura3 SAC6*$^+$	AAB184 (L)
AAY1438	*MAT***a** *his3 leu2 lys2 ura3 sac6*::*LEU2*	AAB184 (L)
AAY1445	*MAT*α *his3 leu2 lys2 ura3 SAC6*$^+$	AAB188 (T)
AAY1446	*MAT*α *his3 leu2 lys2 ura3 sac6*::*LEU2*	AAB188 (T)
AAY1635	*MAT***a** *his3 leu2 lys2 ura3 sac6*::*LEU2*	AAB268 (I)
AAY1637	*MAT***a** *his3 leu2 lys2 ura3 SAC6*$^+$	AAB268 (I)
AAY1639	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 SAC6*$^+$/*SAC6*$^+$	AAB268 (I)
AAY1640	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 SAC6*$^+$/*SAC6*$^+$	AAB268 (I)
AAY1641	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 sac6*::*LEU2*/*sac6*::*LEU2*	AAB268 (I)
AAY1642	*MAT***a**/*MAT*α *ade2*/+ *his3*/*his3 leu2*/*leu2 lys2*/*lys2 ura3*/*ura3 sac6*::*LEU2*/*sac6*::*LEU2*	AAB268 (I)

[a] AAY1069 is heterozygous for the *sac6*::*LEU2* null mutation, which was generated by precise replacement of the entire *SAC6* coding sequence with the yeast *LEU2* gene (3). AAY1047 and AAY1048 are haploid segregants from this diploid. AAY1069 was transformed with pTS161, pTS162, AAB184, AAB186, AAB188, AAB190, or AAB268. Haploid strains listed, other than AAY1047 and AAY1048, are all segregants derived by tetrad dissection from these transformed diploids. Diploid strains other than AAY1069 were all obtained by crossing in various combinations segregants derived from the transformed diploids. All strains listed, both haploid and diploid, are Gal$^+$. The plasmids contained within the various strains are described in Materials and Methods.

ACT1 or *ARF1* termination sequence (27). Chicken fimbrin (1,893 bp) (8) and human I-fimbrin (1,890 bp) (18) coding sequences were cloned into an *Escherichia coli* pABX-based plasmid (25), from which they were isolated by *Bam*HI and *Xba*I digestion and subcloned into pTS161 as described above. Upstream of the ATG initiation codon were ACAT and a *Bam*HI recognition site. Downstream of the stop codon were *Eco*RI and *Xba*I recognition sites. The human fimbrin-containing plasmids are designated AAB184 (L-fimbrin in pTS161), AAB186 (L-fimbrin in pTS162), AAB188 (T-fimbrin in pTS161), AAB190 (T-fimbrin in pTS162), and AAB268 (I-fimbrin in pTS161).

Test of the ability of human fimbrins to complement the *sac6* null defect. Plasmids AAB184, AAB186, AAB188, AAB190, and AAB268 and the parent vectors pTS161 and pTS162 (see above) were used to transform yeast strain AAY1069 (*sac6*::*LEU2*/+ *ura3*/*ura3* [Table 1]). Ura$^+$ transformants were selected and sporulated, and tetrads were dissected on YEP-glucose plates. Segregants were tested for growth in the absence of leucine (to identify segregants carrying the *sac6*::*LEU2* null mutation) or uracil (to identify segregants carrying the *URA3*-containing plasmid). Segregants were also tested for growth at 25 and 37°C on rich (YEP) medium under repressing (glucose) or inducing (galactose) conditions. Representative segregants of each genotype were used for the composite plate shown in Fig. 1. Diploids generated by crosses of haploid segregants were used to obtain quantitative measurements of growth rates of strains carrying the various fimbrin isoforms (Table 2) as well as to test for complementation of the defects in sporulation (Table 3), cell shape (Fig. 3), and organization of the cytoskeleton (Fig. 3). These diploids are listed in Table 1. Complementation by chicken fimbrin was tested by transforming strains AAY1047 and AAY1048 (Table 1) with the chicken fimbrin plasmid construct (see above), selecting Ura$^+$ transformants, and testing for growth on rich medium containing glucose or galactose as described above.

Determination of growth rates. Diploid yeast strains listed in Table 2, containing plasmids with or without various isoforms of human fimbrin, were struck out on uracil-deficient plates to select for plasmid, and single colonies were patched to YEP-galactose plates at 30°C for 3 days. Cells were then inoculated into selective liquid medium lacking uracil with 2% glucose or 2% galactose (U.S. Biochemical catalog no. 15996) and incubated at 23°C. When cultures were in mid-log phase, they were diluted to about 10^6 cells per ml in the same medium at ~23°C and incubated for 2 h. Half of each culture was then shifted to 37°C, the other half remaining at ~23°C. Samples were taken from each culture (glucose or galactose at ~23 or 37°C) at 1-h intervals for about 6 h. Samples obtained throughout the experiment were fixed and sonicated before cell numbers were determined with a Coulter Counter (23). Generation times were calculated for each strain under the various conditions and are listed in Table 2.

Sporulation. To test whether the various fimbrin isoforms were able to complement the sporulation defect of *sac6*/*sac6* mutant strains (see Results and Discussion), homozygous *SAC6*$^+$/*SAC6*$^+$ wild-type and *sac6*::*LEU2*/*sac6*::*LEU2* mutant strains containing plasmids with T-, L-, or I-fimbrin or no fimbrin were grown in medium lacking uracil (to select for a plasmid) under either inducing (galactose) or noninducing (glucose) conditions at 23°C. Early-stationary-phase cells were then transferred to sporulation medium (1% potassium acetate, 0.05% glucose, 0.1% yeast extract) at 23°C and were examined for sporulation after several days.

Fluorescence microscopy. Diploid cells were grown in suspension at 23°C in synthetic medium without uracil (to select for a plasmid) or rich nonselective medium (YEP), using raffinose as the carbon source. When cells reached log phase, fimbrin expression was induced by addition of galactose for 4 h. Rhodamine-phalloidin staining of actin was carried out as described elsewhere (25) by a modification of a procedure described previously (5). The stained cells were flattened by hand pressing against a metal block and examined under a Zeiss Axioskop with a 100× objective. Initial observations of wild-type cells indicated that cells grown on synthetic medium containing galactose had less obvious actin cables than those grown on rich medium containing galactose. However, when mutant cells containing fimbrin plasmids were grown on rich medium, a high percentage of cells appeared to lose plasmids (as judged by the presence of many mutant-looking cells in the stained population). The experiments described here were therefore carried out on cells grown in selective medium containing galactose. All observations and cell counts described in the text were made with such cells. For the purposes of illustration, however, Fig. 3 shows wild-type and mutant cells containing vector alone (strains AAY1415 and AAY1416) that had been grown in rich medium (YEP) with glucose.

Detection of the human fimbrin isoforms in yeast cells by immunoblotting. To determine whether the three human fimbrins were properly expressed in yeast cells, 50-ml cultures of strains AAY1416, AAY1420, AAY1423, and AAY1641 (Table 1) were grown at room temperature to a density of 5×10^6 cells per ml in uracil-deficient synthetic medium with 2% raffinose as the carbon source. The cultures were then induced by adding galactose to a concentration of 2% and grown for an additional 5 h before being harvested. In each case, yeast cells at 6 units of optical density at 600 nm were lysed with 200 μl of 1.85 M NaOH and

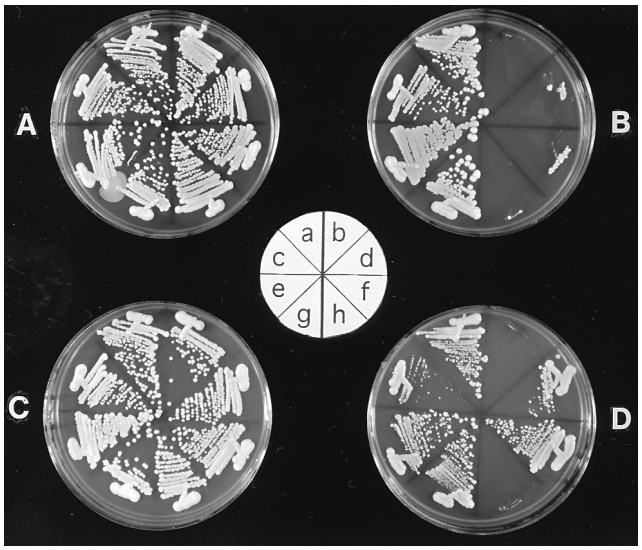

FIG. 1. Complementation of the yeast *sac6* temperature-sensitive growth defect by human T- and L-fimbrins. *SAC6*$^+$ wild-type (on the left half of each plate) or *sac6*::*LEU2* null (on the right half of each plate) strains carrying plasmids with no fimbrin (a and b), human T-fimbrin (c and d), human L-fimbrin (e and f), or human I-fimbrin (g and h) were incubated on rich medium containing glucose (plates A and B) or galactose (plates C and D) for 2 days at 25°C (plates A and C) or 37°C (plates B and D). Strains: a, AAY1429; b, AAY1430; c, AAY1445; d, AAY1446; e, AAY1437; f, AAY1438; g, AAY1637; h, AAY1635. Genotypes of strains are listed in Table 1.

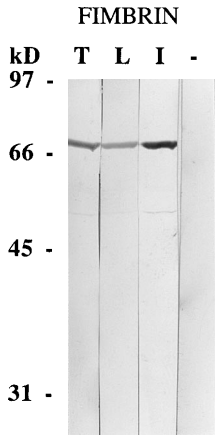

FIG. 2. Immunodetection of three human fimbrin proteins expressed in *sac6* mutant yeast cells. Lanes T, L, I, and − represent total yeast lysates from strains AAY1423 (T-fimbrin), AAY1420 (L-fimbrin), AAY1641 (I-fimbrin), and AAY1416 (no fimbrin), respectively. The antifimbrin antibody R163.3 was used to detect the various isoforms. The high intensity of the I-fimbrin band reflects the preference of the antibody for I-fimbrin over T- and L-fimbrins because of the fact that it was originally raised against chicken intestinal fimbrin. Molecular weight markers are indicated on the left.

7% β-mercaptoethanol for 10 min at 4°C. An equal volume of 50% trichloroacetic acid was added, and the mixture was incubated for an additional 10 min at 4°C. The protein pellet was subsequently collected by spinning for 10 min at 4°C in an Eppendorf centrifuge and resuspended in 2× sample buffer (200 mM dithiothreitol, 4% sodium dodecyl sulfate [SDS], 125 mM Tris [pH 6.8], 20% glycerol, 0.006% bromophenol blue). After neutralization with 1 M Tris base, 5 μl of the sample was analyzed by SDS-polyacrylamide gel electrophoresis. Rabbit polyclonal antibody R163.3 (10), which specifically recognizes human T-, L-, and I-fimbrins, but not yeast fimbrin, was used for immunoblotting as described previously (10).

RESULTS AND DISCUSSION

Complementation of the temperature-sensitive growth defect of the *sac6* null mutant. To test whether yeast and human fimbrins are functionally conserved in vivo, we placed the genes encoding the human proteins under the control of an inducible promoter and examined whether expression of these genes could complement the *sac6* null defect. Thus, the human T- L-, and I-fimbrin genes were placed under the control of the yeast *GAL1* promoter, and the resulting plasmids were used to transform *SAC6*$^+$ and *sac6* null mutant cells (see Materials and Methods). If the human fimbrin genes could functionally substitute for yeast fimbrin, the transformants should have been able to grow at 37°C on galactose.

The key result was that at 37°C, those *sac6*::*LEU2* segregants containing either T- or L-fimbrin, but not the control plasmids or I-fimbrin, grew well on galactose (Fig. 1D). Moreover, at 37°C, the *sac6*::*LEU2* segregants, with or without human fimbrin, failed to grow on glucose (Fig. 1B), indicating that growth requires transcription from the *GAL1* promoter. Failure of the I-fimbrin to complement the defect is not due to inability of this isoform to be expressed in yeast cells, as immunoblotting shows that T-, L-, and I-fimbrins are all induced (Fig. 2). To further confirm that I-fimbrin, a specialized intestinal microvillar fimbrin, was unable to complement the yeast *sac6* null mutation, a functionally equivalent chicken I-fimbrin was also tested for complementation (see Materials and Methods). Chicken intestinal fimbrin also failed to complement the defect (not shown).

At 25°C, all wild-type and mutant segregants, with or without human fimbrin, grew well on rich medium containing either glucose or galactose (Fig. 1A and C). At 37°C, all wild-type segregants, with or without human fimbrin, grew well on either glucose or galactose (Fig. 1B and D). This finding indicates that the human fimbrins do not have dominant negative phenotypes. Together, these results demonstrate that human

TABLE 2. Generation times of wild-type and *sac6* mutant strains in which various human fimbrin isoforms are or are not expressed[a]

Strain	*SAC6*	Fimbrin	Generation time (h)			
			Glucose		Galactose	
			23°C	37°C	23°C	37°C
AAY1417	+	None	5.4	3	3	3.7
AAY1415	+	None	2.7	2.7	3.3	3.5
AAY1418	−	None	3.4	>25	3.9	~15
AAY1416	−	None	3.3	>25	5	>25
AAY1422	+	L	2.4	2	4.1	2.9
AAY1419	+	L	2.4	2.2	4.2	4.7
AAY1421	−	L	3.4	>25	3.6	3.1
AAY1420	−	L	3.2	>25	5.8	6.2
AAY1426	+	T	2.6	1.8	4.1	2.9
AAY1424	+	T	2.4	1.8	3.9	3.6
AAY1425	−	T	3.4	>25	4.1	3.8
AAY1423	−	T	3.2	>25	4.2	4.8
AAY1639	+	I	2	1.7	3.8	2.8
AAY1640	+	I	2.1	1.9	3.7	3.8
AAY1641	−	I	3.2	>25	4.2	>25
AAY1642	−	I	2.9	>25	4.6	>25

[a] Genotypes of strains used are listed in Table 1. Except for those strains containing I-fimbrin, the first strain of each pair listed contains a plasmid whose parent vector was pTS162, and the second strain of the pair contains a plasmid whose parent vector was pTS161 (see Materials and Methods). In the case of the strains containing I-fimbrin, the plasmid in all four cases was derived from the pTS161 parent. Generation times were determined as described in Materials and Methods.

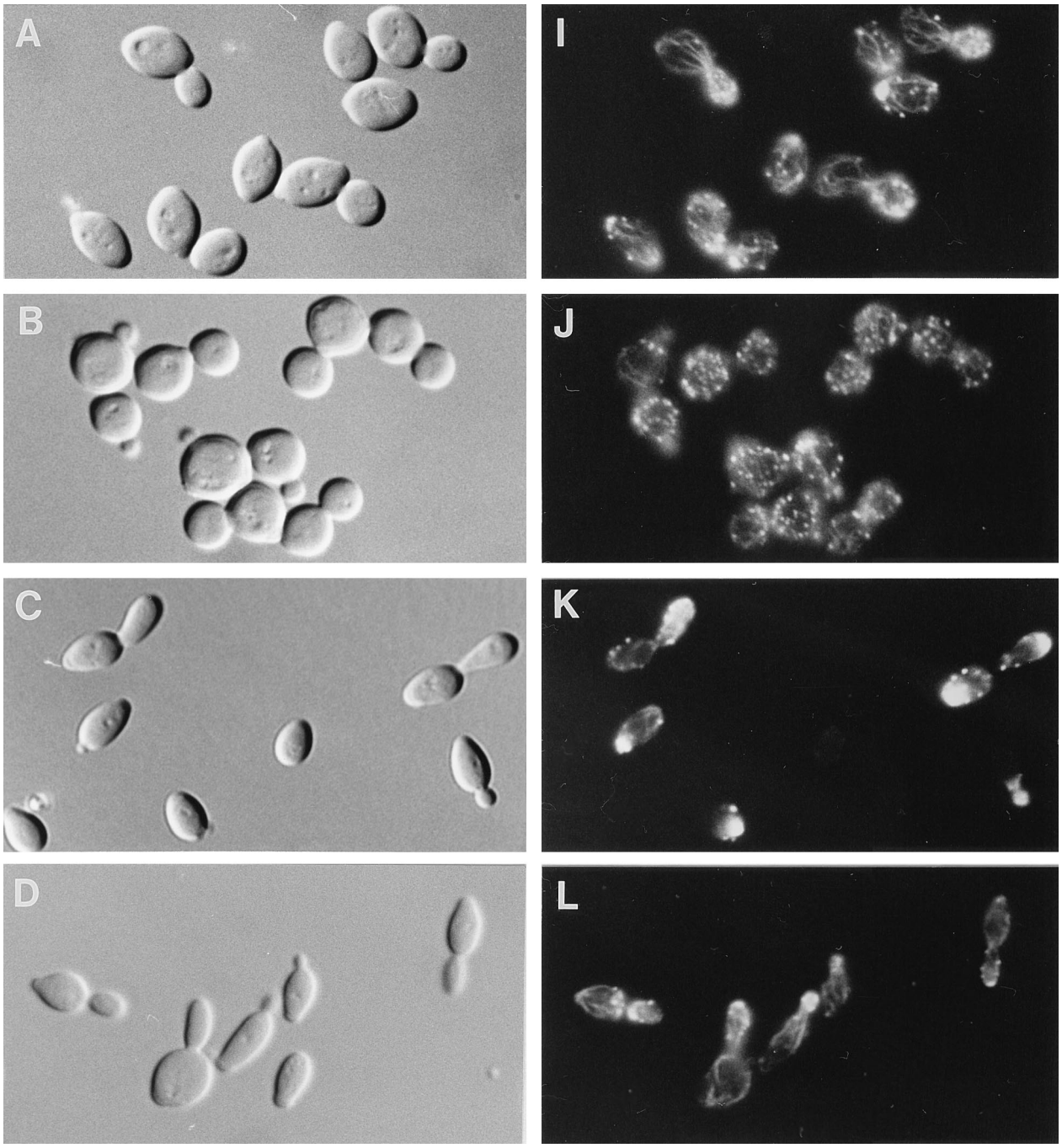

FIG. 3. Complementation by human T- and L-fimbrins, but not I-fimbrin, of the defects in cell shape (Nomarski optics micrographs [A to H]) and cytoskeletal organization (fluorescence micrographs [I to P]) caused by the *sac6* null mutation at 23°C. *SAC6*$^+$/*SAC6*$^+$ wild-type strains (A, C, E, G, I, K, M, and O) and *sac6*/*sac6* mutant strains (B, D, F, H, J, L, N, and P) containing plasmids with no fimbrin (A, B, I, and J), L-fimbrin (C, D, K, and L), T-fimbrin (E, F, M, and N), or I-fimbrin (G, H, O, and P) were grown on rich (YEP) medium containing glucose (A, B, I, and J) or uracil-deficient synthetic medium containing galactose (all other panels) at 23°C. Strains used are the same as those listed in Table 3.

T- and L-fimbrins, but not I-fimbrin, can complement the temperature-sensitive growth defect in *sac6* mutant cells.

The extent of complementation by the various fimbrin isoforms was determined quantitatively from an analysis of the growth rates of various strains. Thus, homozygous wild-type or mutant diploid strains with plasmids containing T-, L-, or I-fimbrin or no fimbrin sequences (Table 2) were grown in suspension at 23 or 37°C under inducing (galactose) or repressing (glucose) conditions as described in Materials and Methods. The growth rates of the various strains under these conditions (Table 2) lead to the same conclusions as were drawn from visual inspection of the plates (Fig. 1). In particular, mutant strains expressing either T- or L-fimbrin grow at rates similar to the wild-type rate at 37°C on galactose but not glucose, whereas mutant strains containing either a vector alone or a plasmid with the I-fimbrin gene fail to grow on either glucose

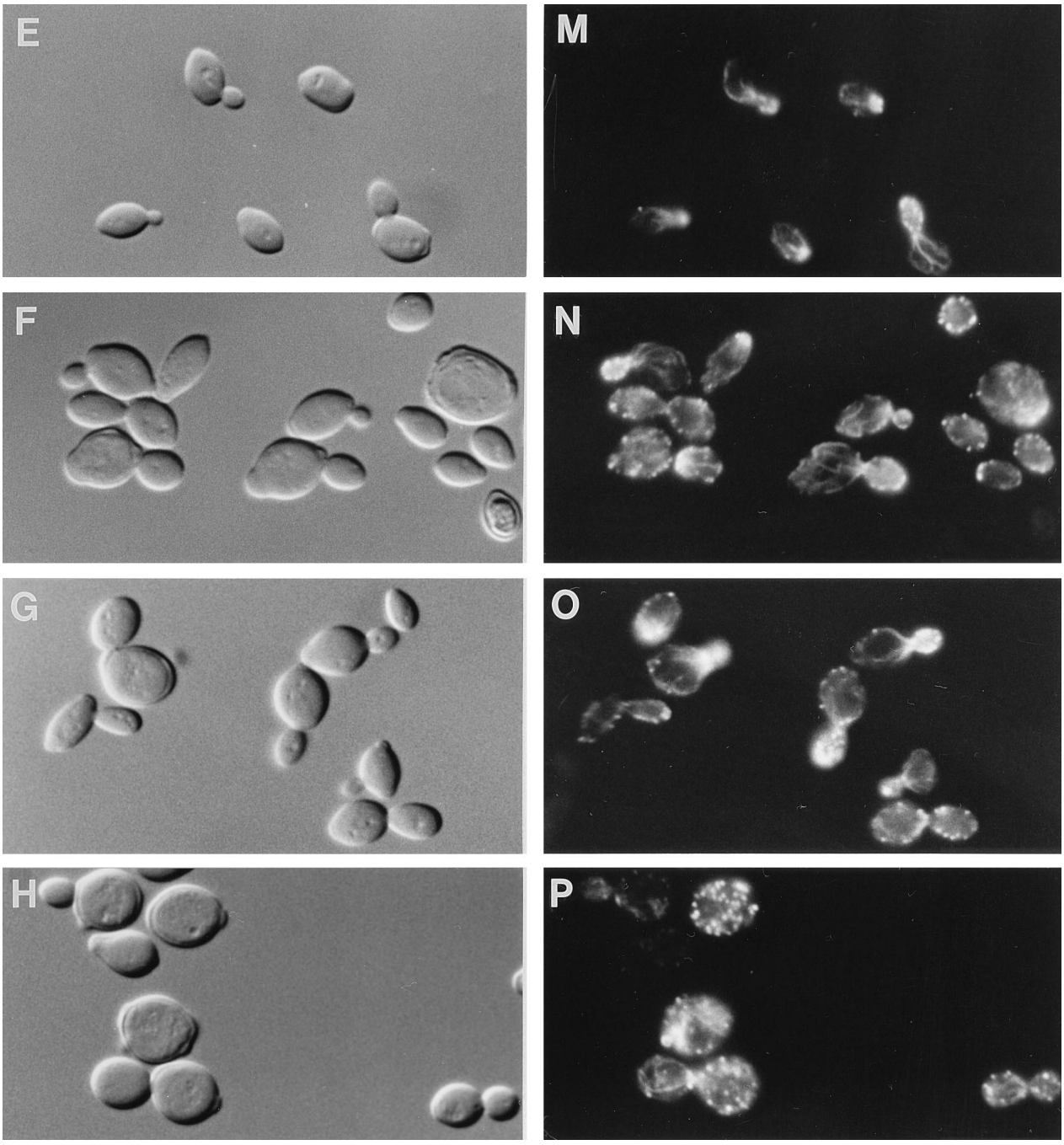

FIG. 3—*Continued.*

or galactose at 37°C. Furthermore, all strains, regardless of genotype, plasmid, or carbon source, grow at 23°C, and expression of human L-, T, or I-fimbrin in *SAC6*$^+$ cells is not noticeably deleterious to the growth rates of the cells under any conditions (i.e., 23 or 37°C, on glucose or galactose [Table 2]).

Complementation of the defect in the organization of the cytoskeleton. Sac6p is a component of the yeast actin cytoskeleton (2, 3, 9), and the temperature-sensitive growth defects seen in mutant cells lacking this protein can be attributed to defects in the organization of the actin cytoskeleton (3, 4). The intracellular distribution of actin in these cells is aberrant, with cytoplasmic actin cables drastically reduced in both number and intensity and the cortical actin dots less asymmetrically distributed than in wild-type cells (3). To determine whether the human fimbrins complement the growth defects through a direct effect on the cytoskeleton, we examined wild-type and mutant yeast cells expressing one of the various fimbrin isoforms or no fimbrin (vector alone) during growth on galactose. When the cells were stained with rhodamine-phalloidin and viewed by fluorescence microscopy, approximately 95% of the cells of strain AAY1416 (*sac6*::*LEU2*/*sac6*::*LEU2*, vector alone) displayed a loss of actin cables and aberrant distribution

of actin patches, similar to those shown in Fig. 3J. In contrast, about 80% of the cells of strain AAY1423 (*sac6*::*LEU2*/*sac6*::*LEU2*; containing T-fimbrin) had visible actin cables and asymmetrically localized actin patches (Fig. 3N). Similar results were obtained with cells expressing L-fimbrin (Fig. 3L), indicating that the normal abundance of actin cables and the asymmetry in actin distribution seen in wild-type cells (on either glucose or galactose [Fig. 3I]) are at least partially restored to the mutant cells containing plasmids with T- or L-fimbrin under inducing conditions. In contrast, mutant cells expressing I-fimbrin (Fig. 3P) are indistinguishable from mutant cells carrying plasmid with no fimbrin (on either glucose or galactose [Fig. 3J]). Expression of any of the three human fimbrins in wild-type yeast cells does not detectably perturb the actin cytoskeleton (compare Fig. 3I with Fig. 3K, M, and O). Thus, human T- and L-fimbrins most likely function in yeast cells through a direct interaction with the actin cytoskeleton, thereby restoring the defects caused by the *sac6* null mutation.

Complementation of the defect in cell morphology. As the human L- and T-fimbrins function in yeast cells through a direct interaction with the actin cytoskeleton, it seemed likely that these human fimbrins would restore the *sac6* null defect in cell shape. Individual cells were therefore examined morphologically. As reported previously (3), *sac6*/*sac6* null mutant diploid cells growing at the nominally permissive temperature of 23°C are abnormally round, rather than ellipsoidal, compared with wild-type cells (Fig. 3A and B). Microscopic examination of *sac6*/*sac6* mutant cells expressing either L- or T-fimbrin, however, revealed that the cells are ellipsoidal when grown on galactose (Fig. 3D and F). In contrast, mutant cells expressing I-fimbrin are round like those containing the vector alone (Fig. 3B and H).

These results demonstrate that both the T and L, but not the I, isoforms of human fimbrin can complement the *sac6* null defect in cell shape.

Complementation of the sporulation defect of *sac6*/*sac6* mutant diploid cells. Analysis of *sac6*/*sac6* homozygous mutant diploid cells reveals a complete defect in sporulation. That is, after several days in sporulation medium (see Materials and Methods), congenic wild-type diploid cells form tetrads as normal, but rarely can even one *sac6*/*sac6* mutant ascus be found when numerous fields are scanned microscopically.

To examine whether any of the human fimbrin isoforms are able to complement the sporulation defect seen in *sac6*/*sac6* mutant cells, wild-type and mutant diploid strains containing T-, L-, or I-fimbrin or no fimbrin were grown to early stationary phase in uracil-deficient selective medium containing either glucose or galactose at 23°C (see Materials and Methods). Cells were then transferred to sporulation medium and incubated at 23°C for several days before being examined microscopically. As shown in Table 3, 29 to 65% of wild-type $SAC6^+/SAC6^+$ cells of different strains were able to sporulate, regardless of either the plasmid they contained or whether they had been grown on glucose or galactose prior to incubation under sporulation conditions. In contrast, *sac6*/*sac6* mutant diploids were generally able to sporulate only if (i) they contained plasmids with the T- or L-fimbrin gene (9 or 7% sporulation, respectively) and (ii) they had been grown under inducing (galactose) conditions prior to incubation in sporulation medium. Mutant cells containing either the vector alone or the vector plus the I-fimbrin gene failed to sporulate regardless of whether they had been grown in glucose or galactose prior to incubation in sporulation medium. These results indicate that both T- and L-fimbrins, but not I-fimbrin, are able to complement the *sac6* null defect in sporulation.

It is notable that the defect in sporulation can be complemented by human fimbrins, which normally function in cells that never sporulate. This observation suggests that the role of Sac6p in sporulation may be more general, and in this light, it will be interesting to determine the mechanistic requirement for Sac6p in sporulation.

TABLE 3. Complementation of the *sac6*/*sac6* defect in sporulation by human fimbrins[a]

Strain	*SAC6*	Fimbrin	% Sporulation	
			Glucose	Galactose
AAY1415	+	None	29	44
AAY1416	−	None	0	0
AAY1419	+	L	65	54
AAY1420	−	L	0	7
AAY1424	+	T	56	54
AAY1423	−	T	0.2	9
AAY1639	+	I	65	56
AAY1641	−	I	0	0

[a] The genotypes of the strains used are listed in Table 1. Five hundred cells were counted under each condition for strains AAY1416, AAY1420, AAY1423, and AAY1641; 200 cells were counted in each case for strains AAY1415, AAY1419, AAY1424, and AAY1639.

Failure of I-fimbrin to complement the *sac6* null defect. The observation that I-fimbrin does not complement any of the *sac6* null defects is intriguing. A trivial explanation would be that the levels of I-fimbrin present in yeast cells are different from those of L- and T-fimbrins, either because of different levels of expression or because of different rates of turnover. We have ruled out the possibility that I-fimbrin is not expressed at all by immunoblotting experiments that show that I-fimbrin is present under inducing conditions at levels roughly similar to those of L- and T-fimbrins (Fig. 2). We cannot rule out the formal possibility that either too much or too little I-fimbrin is responsible for the failure to complement the *sac6* defect. However, a more interesting possibility is that there is a functional difference between the different fimbrins and that this difference can be detected even in yeast cells. Indeed, the L-, T-, and I-fimbrin isoforms have been shown to have specificity in both tissue localization (18) and subcellular localization (see reference 25 and references cited therein): I-fimbrin is found in the brush border of intestinal epithelial cells, L-fimbrin is present in focal adhesions of leukocytes, and T-fimbrin is found in the leading membrane and focal adhesion sites of many other cell types. Furthermore, the three fimbrin isoforms seem to have different specificities in their binding to actin isoforms. I-fimbrin is found in microvilli, which have specialized actin isoforms (24), L-fimbrin binds strongly to β-actin (22) and only weakly to muscle actin (21, 22), and T-fimbrin binds more strongly to muscle actin (21). The functional difference uncovered for I-fimbrin in *S. cerevisiae* may reflect previously unrecognized functional differences in human fimbrins. We do not know whether the three human isoforms differentially localize to actin structures in yeast cells because at least in the case of T-fimbrin, immunofluorescence experiments were uninformative as a result of high levels of background staining, presumably the result of high levels of overexpressed protein. However, using our complementation assay, we should be able to identify the region of I-fimbrin that is responsible for these functional differences.

In conclusion, several observations indicate that T- and L-fimbrins are functionally interchangeable with the yeast homolog. First, temperature-sensitive lethality is complemented. Second, the cell shape is restored. Third, the actin cytoskeleton is organized as in wild-type cells. Fourth, the sporulation defect

is reversed. The ability of the human proteins to interact with the yeast machinery, to the extent that the mutant cells are essentially indistinguishable from wild-type cells, reveals a remarkable level of conservation between the human and yeast proteins. This finding sets the stage for a genetic analysis of the human proteins in vivo, using the powerful tools possible with *S. cerevisiae* to genetically manipulate the genes, replace the yeast *SAC6* gene with the altered human genes, and analyze the activities of the mutant protein in cells in which this is the sole source of fimbrin. Preliminary results from such an analysis are described elsewhere (25).

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