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Different steps of sexual development are differentially regulated by the Sec8p and Exo70p exocyst subunits.

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Abstract

Here we show that in *Schizosaccharomyces pombe* mating-specific cell adhesion is dependent on the exocyst subunit Sec8p but independent of the exocyst subunit Exo70p. In the absence of Exo70p, the forespore membrane does not develop properly and the leading edge protein Meu14p is abnormally distributed. Additionally, the spindle pole body is aberrant in a significant number of *exo70Δ* asci. In both, the *sec8-1* and the *exo70Δ* mutants the development of the spore cell wall is impaired. These results show that different steps of sexual development are differentially regulated by the exocyst and suggest the existence of exocyst sub-complexes with distinct roles in mating.

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1. Introduction

Schizosaccharomyces pombe cells belong to either of two mating types: h^+ or h^-. Homothallic h^{90} strains are self-fertile because a mating-type switching allows them to form colonies containing h^+ and h^- cells. When nitrogen is scarce, the Ste11p transcription factor induces the expression of genes essential for sexual development, including those coding for pheromones (Yamamoto, *et al.*, 1997, Nielsen, 2004, Shimoda & Nakamura, 2004, Yamamoto, 2004). The binding of these pheromones to receptors in cells of the opposite mating type initiates a signalling pathway that requires a mitogen-activated (MAP) kinase cascade consisting of Byr2p, Byr1p and Spk1p. As a result, cells differentiate into shmoos with a polarized growth pattern (Nielsen, 2004). Then the Mam3p and Map4p agglutinins (Yamamoto, *et al.*, 1997, Mata & Bahler, 2006, Sharifmoghadam, *et al.*, 2006) facilitate and strengthen the union of the shmoos, producing prezygotes (Calleja & Johnson, 1971). Later on, the cell walls between the two mating partners degrade, allowing fusion of the membranes, diffusion of the cytoplasmic material and karyogamy producing a diploid zygote (Calleja, *et al.*, 1977, Nielsen, 2004, Yamamoto, 2004).

The diploid nucleus immediately undergoes meiosis and gives rise to four haploid nuclei (Shimoda & Nakamura, 2004, Yamamoto, 2004). The leading edge protein (LEP) Meu14p accumulates besides the spindle pole body (SPB), which acts as a centre for the organization of the forespore membrane (FSM), and forms ring-shaped structures that promote the development of the membrane around the nuclei (Ikemoto, *et al.*, 2000, Okuzaki, *et al.*, 2003, Shimoda, 2004, Shimoda & Nakamura, 2004, Nakamura, *et al.*, 2008, Nakase, *et al.*, 2008). FSM development requires the secretory pathway and vesicle docking (Shimoda, 2004, Shimoda & Nakamura, 2004, Nakamura, *et al.*, 2008). Spores maturate with the building of a specialized cell wall, which involves the synthesis of α- and β-glucan and chitin (Arellano, *et*

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al., 2000, Liu, *et al.*, 2000, Martín, *et al.*, 2000, Matsuo, *et al.*, 2005, Garcia, *et al.*, 2006, de Medina-Redondo, *et al.*, 2008).

The exocyst is a protein complex involved in the tethering and spatial targeting of post-Golgi vesicles to the plasma membrane before vesicle fusion (TerBush, *et al.*, 1996, Guo, *et al.*, 1999, Mehta, *et al.*, 2005). In *S. pombe*, this complex participates in cell separation because it is required to target hydrolytic enzymes to the septum (Wang, *et al.*, 2002, Martin-Cuadrado, *et al.*, 2005). The only viable exocyst mutants in this organism are *sec8-1* and *exo70Δ* (Wang, *et al.*, 2002, Wang, *et al.*, 2003). The term exomer refers to a *Saccharomyces cerevisiae* coat complex required for the transport of certain membrane proteins from the trans-Golgi network to the plasma membrane (Wang, *et al.*, 2006, Barfield, *et al.*, 2009). The exomer subunit Chs5p is required for chitin synthesis and mating (Santos, *et al.*, 1997). In *S. pombe,* the Chs5p-homologue Cfr1p is required for cell wall digestion during mating (Cartagena-Lirola, *et al.*, 2006).

The analysis of how cell wall-modifying enzymes required for sexual development reach the cell surface is not only interesting for the characterization of the mating process in yeast, but also represents a model system to study intracellular trafficking during a developmental process. The initial goal of this work was to study the regulation of cell adhesion by genes that have already been implicated in the mating and/or cell wall remodelling processes. To do so we analyzed agglutination in several mutants; the mutants selected were *spk1Δ* (defective in the mating signal transduction pathway; Nielsen, 2004), *spm1Δ* (deleted for a MAP kinase that regulates morphogenesis, cell integrity, and mating; Zaitsevskaya-Carter & Cooper, 1997), *dni1Δ* (deleted for a claudin-like tetraspan protein required for cell wall reorganization and membrane fusion during mating; Clemente-Ramos, *et al.*, 2009), *cfr1Δ* (deleted for an

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exomer component; Cartagena-Lirola, *et al.*, 2006), *sec8-1* (bearing a point mutation in *sec8*$^{+}$; Wang, *et al.*, 2002), and *exo70Δ* (deleted for *exo70*$^{+}$; Wang, *et al.*, 2003). Surprisingly, our results showed that agglutination is dependent on Sec8p but independent of Exo70p. This result prompted us to analyze in detail the role of these exocyst subunits in mating. Our results suggest that Sec8p and Exo70p participate in different sub-complexes which are differentially required during sexual development.

2. Materials and methods

2.1. Strains and growth conditions

All techniques for *S. pombe* growth and manipulation have been described elsewhere (http://www.biotwiki.org/bin/view/Pombe/NurseLabManual). The relevant genotype of the strains used is listed in Table 1 (supplemental material).

2.2. Genetic manipulations

All the tagged proteins were integrated into the chromosome under the control of their own promoters. The combination of mutated alleles with Green Fluorescent Protein (GFP)-tagged proteins was performed either by plasmid transformation or by "random spore" selection from genetic crosses. Exo70p was tagged at its chromosomal locus as described before (Bähler, *et al.*, 1998) using the oligonucleotides: eexo70up (5'-tatatcaaatttacaaaggctgatttagattcttttattacaagcgcgtttgctccttccctacggatccccgggttaattaa-3') and eexo70do (5'-caatatttagtgggtagcttactcgtaagcagaatctgagcagggtaaacaacaaagtcatcaaaaaaggggaggaattcgagctcgtttaaa-3') and a plasmid bearing the Red Fluorescent Protein (RFP; a generous gift from P. Perez).

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2.3. Agglutination and sporulation analyses

Homothallic h^{90} strains were used. Agglutination was performed in liquid minimal medium without nitrogen and mating efficiency was calculated from cultures that had been induced to mate on SPA plates for 15 hours, as described before (Arellano, *et al.*, 2000, Sharifmoghadam, *et al.*, 2006, Sharifmoghadam & Valdivieso, 2008). Since the efficiency of sexual adhesion and sporulation is reduced at temperatures above 28ºC (Clemente-Ramos, *et al.*, 2009 and our unpublished results), the experiments were performed at 32ºC, a semi-restrictive temperature for the *sec8-1* mutant. The agglutination index (AI) was calculated as the $1/OD_{600}$ of the culture supernatant (Sharifmoghadam & Valdivieso, 2008).

2.4. Microscopy

Hoechst 33258 was used for nuclear staining. Images were captured with a Leica DM RXA microscope equipped with a Photometrics Sensys CCD camera, using the Qfish 2.3 program.

2.5. Western blotting

Western blotting was performed as described (Sharifmoghadam & Valdivieso, 2008). 7.5% polyacrylamide gels were used. The α-HA and α-Cdc2 antibodies were used at 1:5000 dilution.

3. Results

3.1. Agglutination depends totally on Spk1p and Sec8p and partially on Spm1p and Cfr1p.

In order to gain information about the requirements for agglutination, the AI of *spk1Δ*, *spm1Δ*, *dni1Δ*, *cfr1Δ*, *sec8-1*, and *exo70Δ* mutants induced to mate in liquid medium was estimated. Wild-type (WT) and *map4Δ* strains were used as controls. As shown in figure 1 A, in the cultures from the *dni1Δ* and *exo70Δ* mutants agglutination took place as efficiently as

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in the WT. In the case of the *cfr1Δ* and *spm1Δ* mutants, the AI was lower than in the WT; however, mating aggregates were observed in these cultures, indicating that agglutination had taken place. The AI for the *spk1Δ* and the *sec8-1* mutants was similar to that of the negative control *map4Δ* (figure 1 A). We then determined whether the agglutination efficiency was correlated with the level of Map4p by observing under the fluorescence microscope cells from the mutants and the WT strain that had been induced to mate in liquid medium. Map4p localizes at the tip of the shmoos and at the mating bridge of the zygotes in the WT strain (Sharifmoghadam, *et al.*, 2006, Sharifmoghadam & Valdivieso, 2008). As shown in figure 1 B, Map4p was readily observed in the *cfr1Δ*, *exo70Δ*, *spm1Δ*, and *dni1Δ* mutants; agglutinin exhibited a weak fluorescent signal in the *sec8-1* cells, and it could not be observed in the *spk1Δ* cells. Western blot analyses were performed to determine the level of Map4p in the cell walls of the WT and the *exo70Δ*, *sec8-1* and *spk1Δ* cells more precisely. As shown in figure 1 C, the level of agglutinin in the cell walls of the WT and *exo70Δ* strains was similar, but it was lower in the *sec8-1* mutant and was undetectable in the *spk1Δ* mutant. These results showed that the MAP kinase Spk1p and the exocyst subunit Sec8p were required for proper Map4p synthesis and delivery to the cell wall, while the exocyst subunit Exo70p was not.

3.2. The sec8-1 and the exo70Δ mutations produce different mating defects.

It has been described that Map4p is required for agglutination in liquid medium and for mating on solid medium (Mata & Bahler, 2006, Sharifmoghadam, *et al.*, 2006, Sharifmoghadam & Valdivieso, 2008). In order to determine whether Sec8p and the Exo70p might play some role in the mating process on solid medium, *sec8-1* and *exo70Δ* cells were induced to mate on SPA plates at 32ºC for 15 hours. Under these conditions, it was found that the mating efficiency (number of zygotes plus asci with respect to the number of zygotes, asci and cells) was 45% for the WT strain, while this value was 6% for the *map4Δ* mutant. As

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described previously (Mata & Bahler, 2006, Sharifmoghadam, *et al.*, 2006), a significant number of shmoos were detected in the *map4Δ* mating mixtures (figure 1 D) and the asci produced by the *map4Δ* mutant had a WT appearance (not shown; Sharifmoghadam, *et al.*, 2006). In the *sec8-1* mutant, mating efficiency was 10%; in the mating mixtures from this mutant a significant number of enlarged shmoos were observed, and about half of the asci contained non-refractile spores with a heterogeneous size (figure 1 D). In the *exo70Δ* mating mixtures, mating efficiency was 42% and mature asci were scarce (less than 10% of the asci contained four refringent spores. Figure 1 D). This phenotype was even more drastic when the cells were induced to mate on solid minimal medium with supplements (under these conditions, no spores could be detected in the *exo70Δ* asci; not shown).

3.3. Exo70p is essential for forespore membrane development but Sec8p is not.

We wished to determine the step in sporulation at which the exocyst was required. Initially, Hoechst staining was performed to determine whether meiosis took place in the *sec8-1* and *exo70Δ* mutant strains. As shown in figures 2 and 3, four nuclei were observed in the asci from both mutants, showing that nuclear division was not defective in the absence of either Sec8p or Exo70p.

Then, we analyzed the development of the forespore membrane (FSM) in the WT, *sec8-1*, and *exo70Δ* strains. To do so, the localization of the syntaxin-like Psy1p was analyzed in the WT strain and in the mutants. As described previously (Nakamura, *et al.*, 2008), in the WT control GFP-Psy1p was observed as cup-shaped structures (figure 2 A i) that developed to form sacs around the nucleus (figure 2 A ii). In the *sec8-1* mutant, the behaviour of Psy1p was similar to that observed in the WT strain (figure 2 B), showing that Sec8p is not required for the development of the FSM. In the *exo70Δ* asci, Psy1p was detected as amorphous

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membranous structures in the cytoplasm of bi-nucleated or tetra-nucleated asci (figures 2 C i and ii) or as vesicle-like or even tubular structures that failed to engulf the nuclei (figures 2 C iii and iv). This result showed that Exo70p is essential for the FSM development.

3.4. Distribution of the leading edge protein Meu14p and the spindle pole body protein Sad1p is abnormal in the absence of Exo70p.

Next, we wished to study in more detail the defect in FSM development exhibited by the exo*70Δ* mutant. To do so, we analyzed the behaviour of the leading-edge protein (LEP) Meu14p in the WT and the *exo70Δ* strains. Cells bearing Meu14p fused to the GFP were induced to mate and observed under the fluorescence microscope. As reported previously (Okuzaki*, et al.*, 2003) and shown in figure 3 A, in the WT strain Meu14p was observed as four rings of different diameters that were situated in the vicinity of the nuclei but apart from them. In the *exo70Δ* asci, the four Meu14p rings seemed to be attached to the nuclei (figure 3 B), suggesting that the LEP complex could not develop properly.

It has been described that in *meu14Δ* mutants, in which the FSM do not develop properly, the spindle pole bodies (SPBs) seem to be fragmented (Okuzaki*, et al.*, 2003). We wished to know whether the same phenomenon was observed in the absence of Exo70p. To do so, asci carrying a Sad1-GFP protein were analyzed under the microscope. We observed that in the mutant strain, 34% of the asci exhibited multiple Sad1-GFP fluorescent dots (figure 3 C), while this value was 11% for the WT strain. This result suggested that the SPBs are unstable in the *exo70Δ* mutant.

3.5. Proper localization of the alpha glucan synthase Mok12p at the spore surface requires both, Sec8p and Exo70p.

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Finally, we analyzed the distribution of the α-glucan synthase-homologues Mok12p and Mok13p, which are required for the synthesis of the spore cell wall. Mok13p is expressed earlier than Mok12p (Garcia*, et al.*, 2006). As reported previously (Garcia*, et al.*, 2006), in the WT strain Mok13p localized to the FSM forming cup-shaped structures and sacs around the nuclei. The same result was obtained for the *sec8-1* mutant (not shown). In the *exo70Δ* mutant, Mok13p formed amorphous structures or small sacs, like those formed by Psy1p, which did not surround the nuclei (not shown). This result was in agreement with an inability of the *exo70Δ* mutant to develop the FSM properly.

The alpha-glucan synthase Mok12p localizes at the surface of the developing spores (Garcia*, et al.*, 2006). Since the spore cell wall is not permeable to Hoechst, we analyzed the localization of the Mok12-GFP protein with respect to the spore surface photographed under a phase contrast microscope. In the control strain, Mok12p was observed at the spore periphery (figure 4; WT). In the *sec8-1* mutant, the distribution of this protein was heterogeneous; in those asci that had refringent spores, Mok12p localized at the spore surface (figure 4; *sec8-1*) while in those asci that exhibited immature spores Mok12p could not be observed. In the *exo70Δ* mutant, the signal corresponding to Mok12p was hardly observed in the asci interior (figure 4; *exo70Δ*). These results suggest that both exocyst subunits participate in the maturation of the spore cell wall.

3.6. Sec8p and Exo70p co-localize partially at the shmoo tip and totally at the spore surface.

All the results described above confirmed that the exocyst was required for mating in *S. pombe* and that different steps of this process are differentially regulated by these exocyst subunits. In order to know whether the different requirements of Sec8p and Exo70p for agglutination and sporulation were a consequence of a different distribution of these proteins,

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cells carrying a GFP-tagged Sec8p and a RFP-tagged Exo70p were induced to mate in liquid medium and were observed under the microscope. As shown in figure 5, both proteins were observed at the cell-cell contact area of the zygotes. However, the localization of both proteins was not the same and their fluorescent signals only overlapped partially in some zygotes (figure 5 A, ii). During sporulation, Sec8-GFP and Exo70-RFP were observed at the surface of the spores. At this localization, the signal from both proteins was mostly overlapping.

4. Discussion

The initial goal of this work was to study the regulation of sexual agglutination by certain genes that have been implicated in mating and/or cell wall remodelling. As expected, we found that the MAP kinase Spk1p, which is necessary for the mating signal transduction pathway (Nielsen, 2004), was required for agglutination. It has been shown that sporulation is retarded in a *spm1Δ* mutant, and it has been suggested that this delay would probably be due to a defect in some event prior to cytoplasmic mixing (Zaitsevskaya-Carter & Cooper, 1997). We have confirmed that in this mutant agglutination indeed proceeds more slowly than in the WT control. A similar defect in agglutination was found in the exomer-defective *cfr1*Δ mutant. In both, the *spm1Δ* and the *cfr1*Δ mutants this slow agglutination was not due to a significant defect in Map4p localization at the cell surface. Thus, Spm1p and Cfr1p must be regulating the h^- agglutinin Mam3p and/or other protein(s) required for agglutination.

In *S. pombe*, the exocyst is necessary for the correct localization of the glucanases required for cell separation during cytokinesis (Martin-Cuadrado*, et al.*, 2005). Here we have shown that exocyst is also required for mating. When we analyzed the role of the exocyst in agglutination, we found that in the *sec8-1* mutant agglutination did not take place and that this

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defect was correlated with a low level of Map4p, although some Map4p could be detected by microscopic observation and by Western blot, suggesting that Sec8p might also regulate other protein(s) required for agglutination. About half of the *sec8-1* asci exhibited abnormal spores, indicating that Sec8p also plays a role in spore development. Surprisingly, in the absence of the Exo70p exocyst subunit Map4p was detected in the cell wall of the mating cells and agglutination was as efficient as in the WT control. These results showed that Sec8p and Exo70p are differentially required for agglutination. A role for some exocyst subunits in the trafficking of adhesion molecules required for synaptic partner choice has been suggested in *Drosophila* (Mehta, *et al.*, 2005). Thus, the participation of exocyst in the regulation of adhesion molecules seems to be a process that is not species-specific.

The defect in sporulation exhibited by the *exo70Δ* mutant was more dramatic than that of the *sec8-1* mutant. Although the possibility that Exo70p might be more necessary for sporulation than Sec8p cannot be ruled out, it is important to take into account that the *sec8-1* mutant carries a point mutation while the *exo70Δ* strain is a null mutant. The function of Sec8p in sporulation seems to be restricted to the last steps of the spore cell wall synthesis since a significant number of asci carry immature spores. In the absence of Exo70p, FSM development was severely impaired and the spore cell wall could not be synthesized. As a consequence, almost no spores could be detected in the *exo70Δ* mating mixtures. In mammalian cells, exocyst components co-precipitate with the plasma membrane t-SNARE syntaxin (Hsu, *et al.*, 1996) and in *S. pombe*, the syntaxin-like protein Psy1p is essential for FSM development (Shimoda, 2004, Shimoda & Nakamura, 2004, Nakamura, *et al.*, 2008). Thus, it is possible that the exocyst-Psy1p interaction is required for the incorporation of new membrane material and/or certain proteins to the developing FSM during sporulation. Additionally, the LEP Meu14p was abnormally distributed in the *exo70Δ* asci. It will be

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interesting to determine whether the exocyst is required for the proper assembly of the LEP complex and, consequently, for FSM development or whether in the absence of the exocyst new membrane material cannot be incorporated to the developing FSM and, as a consequence, the LEP complex cannot develop properly and cannot encircle the nuclei.

In the *meu14Δ* mutant, the SPBs are unstable and appear to be fragmented, which indicates that Meu14p plays a role in spindle pole body stability (Okuzaki, *et al.*, 2003). In the *exo70*Δ mutant, a significant percentage of SPBs were fragmented, even though these cells carried Meu14p. In mammalian cells, Exo70p associates with microtubules, microtubule-organizing centres, and centrosomes (Xu, *et al.*, 2005). Thus, it is possible that in yeast the exocyst might play a direct role in spindle pole body stability during sporulation. However, the fact that in the *exo70Δ* mutant the defect in the FSM development was stronger than the defect in the SPBs suggests that the main function of Exo70p is to contribute to FSM development. These results suggest that FSM development has an influence on the stability of the SPBs and that the different steps in spore development are inter-regulated.

In *S. cerevisiae*, the exocyst localizes specifically to the sites of active secretion and cell growth, where it mediates the secretion of certain proteins (He, *et al.*, 2007). Additionally, the Sec8p exocyst subunit is required for sporulation at a post-meiotic step (Neiman, 1998), although the specific role of Sec8p in this process it is not known. The data presented in this work show that the exocyst plays a role in sexual development in both yeasts. In *S. pombe*, Sec8p and Exo70p localize to the septal area during vegetative growth (Wang, *et al.*, 2002). However, deletion of *sec8*$^{+}$ is lethal while deletion of *exo70*$^{+}$ is not (Wang, *et al.*, 2002, Wang, *et al.*, 2003), which indicates a different requirement for these exocyst subunits during vegetative growth. We have found that agglutination requires Sec8p but not Exo70p, Exo70p

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but not Sec8p is essential for FSM development, and that both, Sec8p and Exo70p are required for proper synthesis of the spore cell wall. Additionally, we have found that Sec8p and Exo70p co-localize at the spore surface but not at the cell-cell contact area in the prezygotes. These results strongly suggest that Sec8p and Exo70p are present in different sub-complexes; one of them (required for agglutination) would lack Exo70p. The fact that Exo70p is also observed at the tip of the contacting shmoos suggests that, although in our experimental conditions we have not observed a mating defect in the *exo70Δ* mutant, this protein might also play some role during the initial steps of mating. A different exocyst sub-complex, carrying Sec8p and Exo70P, would be required for sporulation. In this sub-complex, the presence of Exo70p seems to be more relevant than the presence of Sec8p for the FSM development.

There is increasing evidence suggesting that different exocyst components play different roles and that there are sub-complexes in the exocyst. Thus, in *Drosophila* it has been shown that exocyst function is divisible so different components play distinct roles. Additionally, different GTPases regulate the activity of this multiprotein complex by interacting with different subunits (Wu*, et al.*, 2008), and the localization of different subunits to the sites of active secretion has different requirements (Zajac*, et al.*, 2005). Thus, exocytosis of specific proteins by the exocyst is subject to a complex regulation. Our results support the notion of different exocyst subunits playing distinct roles in some developmental processes in a variety of organisms, from unicellular eukaryotes to metazoa.

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7. Figure legends

Figure 1. Role of the exocyst in mating. A. Agglutination index in cultures from the indicated strains. Samples were taken at the indicated times and the agglutination index was calculated. The experiment was performed three times and a representative result is shown. B. Localization of the Map4-GFP protein in cells from the indicated strains. C. α-HA Western blot of cell wall proteins from the indicated strains expressing an HA-tagged Map4 protein. D. Micrographs of cells from the indicated strains that had been induced to mate on solid medium. The right-hand panel in the *sec8-1* image has been enlarged x 2 to allow a better observation of the spores. Bar, 10 μm.

Figure 2. FSM is aberrant in *exo70Δ* asci. A-C. Nuclear (Hoechst) and FSM (GFP-Psy1) morphology in wild-type (A), *sec8-1* (B), and *exo70Δ* (C) asci. i to iv show different fields from the same culture.

Figure 3. Meu14p and SPBs are abnormal in the ex*o70Δ* mutant. Nuclear (Hoechst) and LEP complex (Meu14-GFP) morphology in wild-type (A) and *exo70Δ* (B) asci. C. Nuclear (Hoechst) and spindle pole body (Sad1-GFP) morphology in an *exo70Δ* ascus.

Figure 4. Distribution of the α(1,3)glucan synthases Mok12p is abnormal in the exocyst mutants. Phase contrast (left panels) and fluorescence (right panels) micrographs of wild-type (upper panels), *sec8-1* (medium panels) and *exo70Δ* (lower panels) asci carrying a Mok12-GFP fusion protein.

Figure 5. Distribution of Sec8p and Exo70p during mating. Sec8-GFP and Exo70-RFP were observed in zygotes (A) and spores (B). i and ii show different fields from the same culture.

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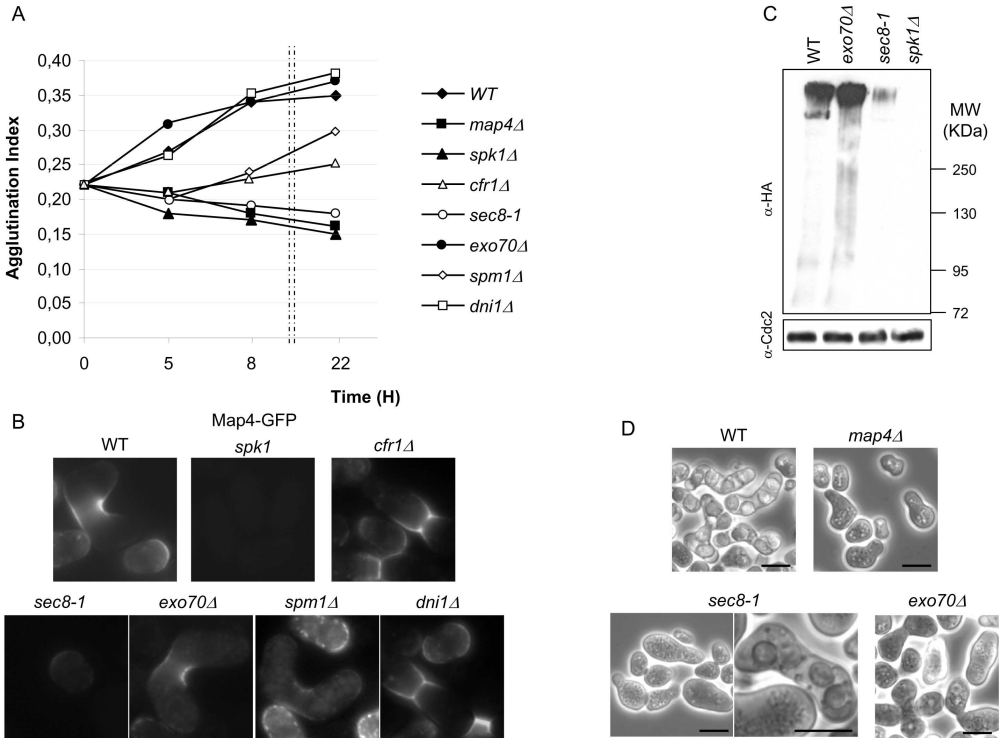

Figure 1. Role of the exocyst in mating. A. Agglutination index in cultures from the indicated strains. Samples were taken at the indicated times and the agglutination index was calculated. The experiment was performed three times and a representative result is shown. B. Localization of the Map4-GFP protein in cells from the indicated strains. C. α-HA Western blot of cell wall proteins from the indicated strains expressing an HA-tagged Map4 protein. D. Micrographs of cells from the indicated strains that had been induced to mate on solid medium. The right-hand panel in the sec8-1 image has been enlarged x 2 to allow a better observation of the spores. Bar, 10 μm.
242x179mm (300 x 300 DPI)

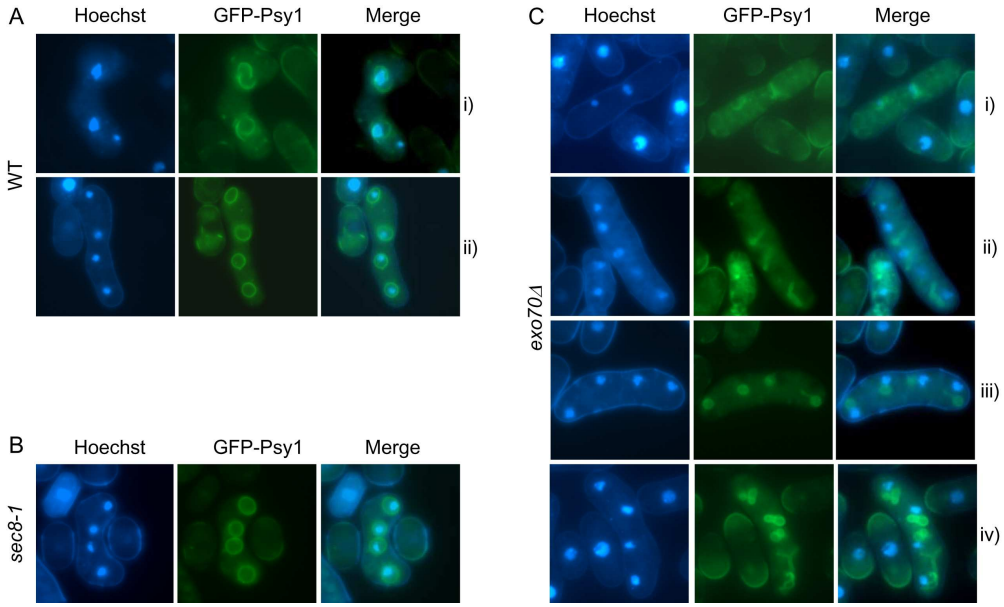

Figure 2. FSM is aberrant in exo70Δ asci. A-C. Nuclear (Hoechst) and FSM (GFP-Psy1) morphology in wild-type (A), sec8-1 (B), and exo70Δ (C) asci. i to iv show different fields from the same culture. 215x129mm (300 x 300 DPI)

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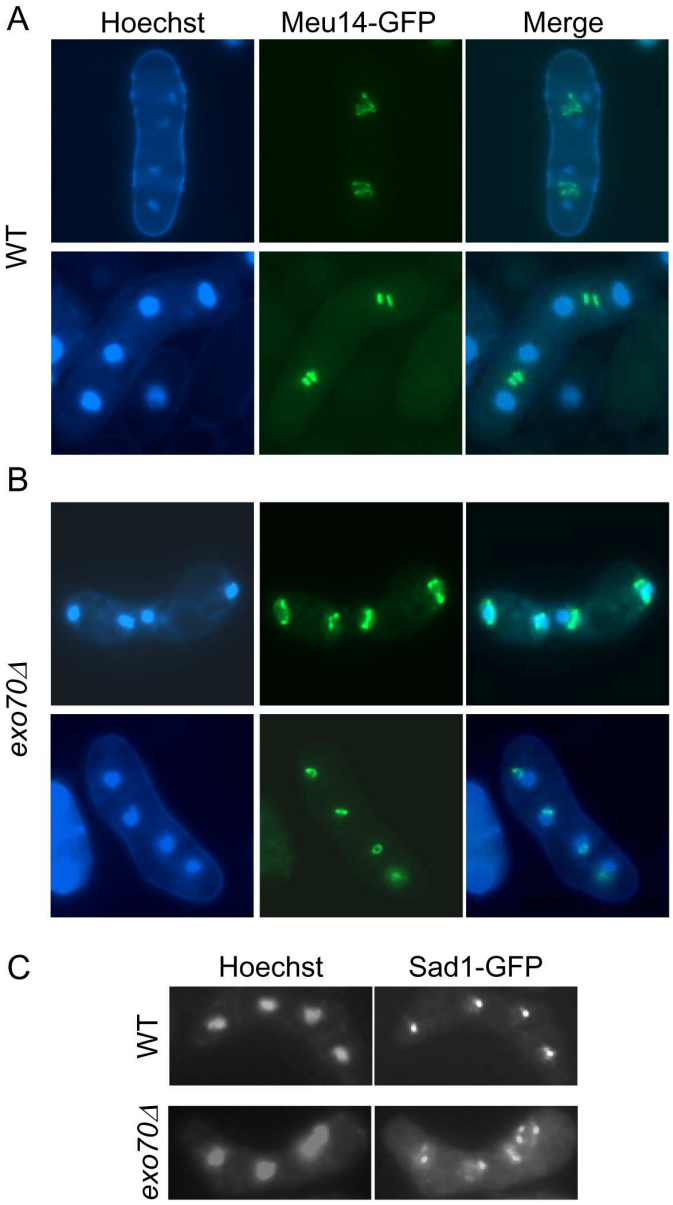

Figure 3. Meu14p and SPBs are abnormal in the exo70Δ mutant. Nuclear (Hoechst) and LEP complex (Meu14-GFP) morphology in wild-type (A) and exo70Δ (B) asci. C. Nuclear (Hoechst) and spindle pole body (Sad1-GFP) morphology in an exo70Δ ascus.
99x177mm (300 x 300 DPI)

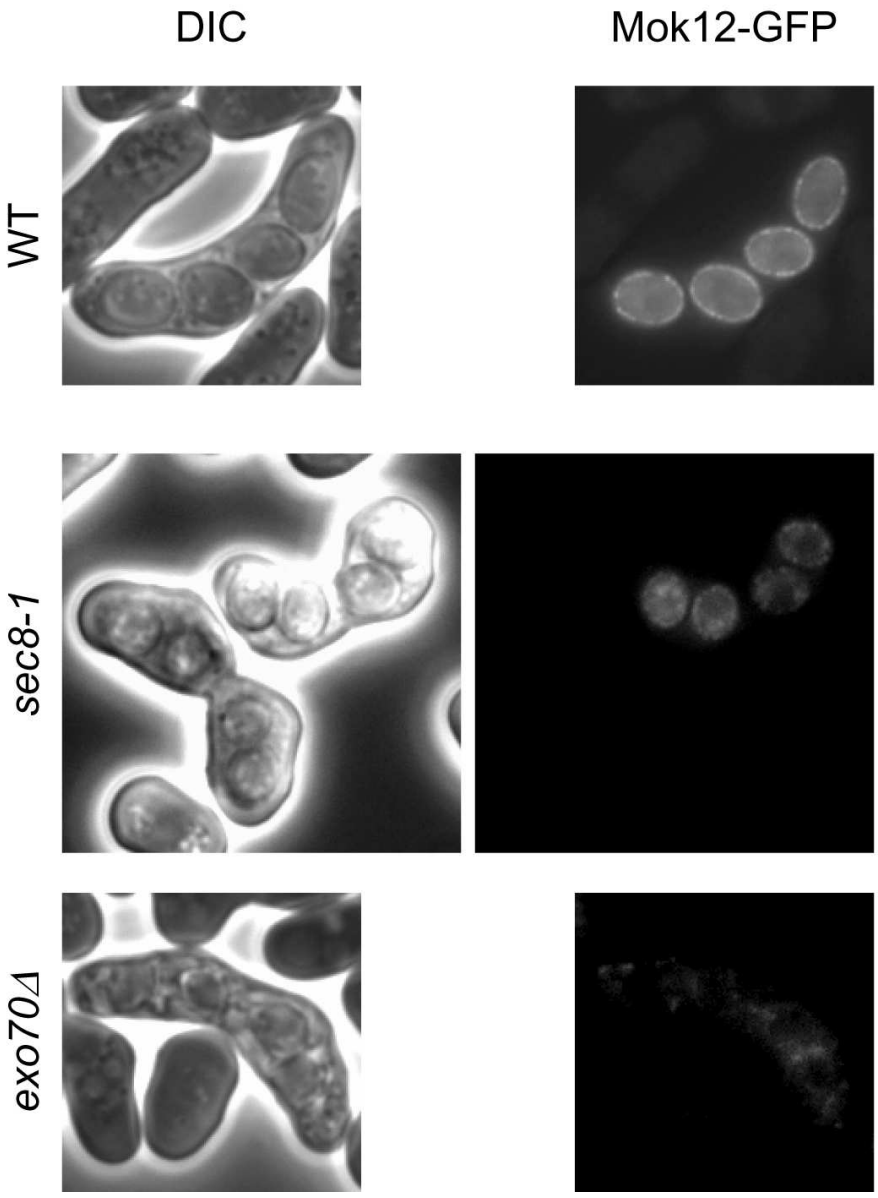

Figure 4. Distribution of the α(1,3)glucan synthases Mok12p is abnormal in the exocyst mutants. Phase contrast (left panels) and fluorescence (right panels) micrographs of wild-type (upper panels), sec8-1 (medium panels) and exo70Δ (lower panels) asci carrying a Mok12-GFP fusion protein.
88x120mm (300 x 300 DPI)

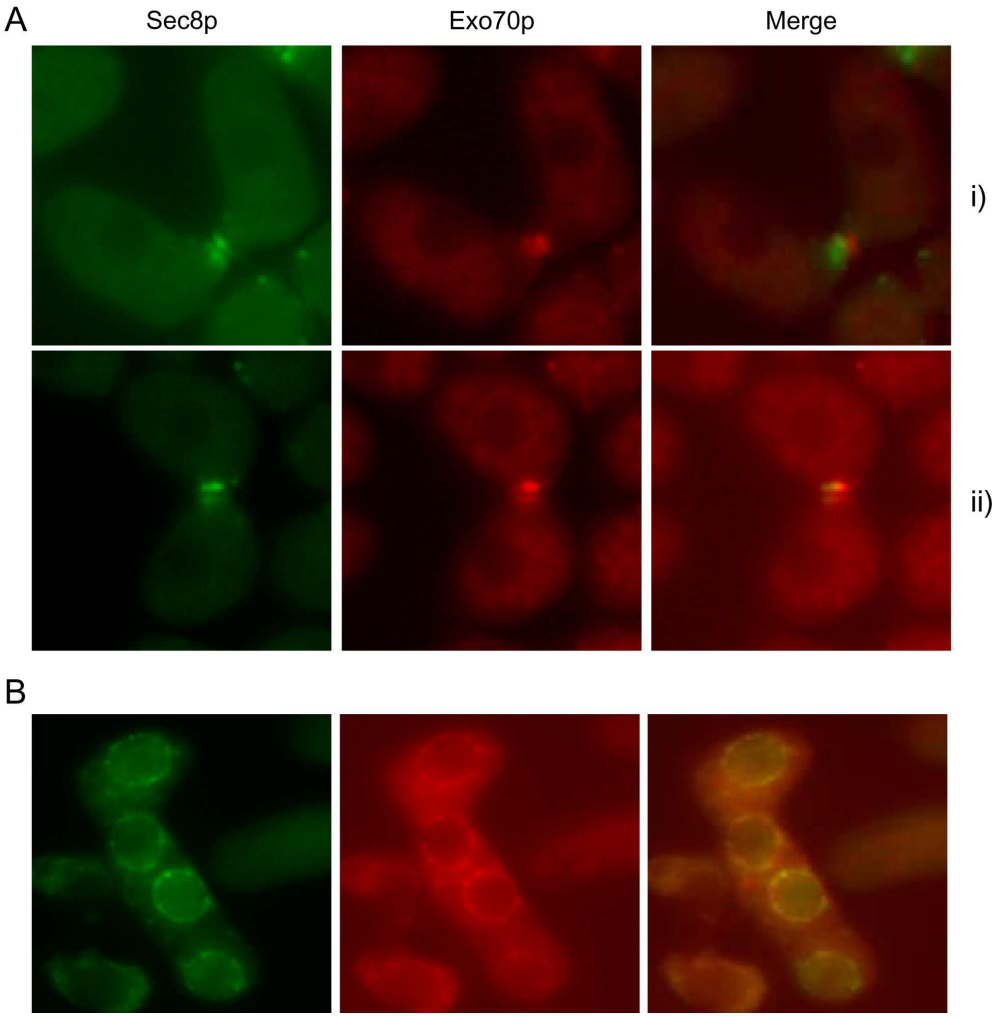

Figure 5. Distribution of Sec8p and Exo70p during mating. Sec8-GFP and Exo70-RFP were observed in zygotes (A) and spores (B). i and ii show different fields from the same culture.
166x168mm (300 x 300 DPI)

Table 1: Strains used in this work

STRAIN	GENOTYPE	SOURCE
HVP366	*h^{90} cfr1::his3^{+} leu1-32 ura4-D18 ade6*	Lab stock
HVP758	*h^{90} dni1::KAN leu1-32 ura4-D18 ade6*	Lab stock
HVP1102	*h^{-} exo70::ura4^{+} leu1-32 ade6*	M. Balasubramanian
HVP1103	*h^{+} sec8-1 ura4-D18 leu1-32*	M. Balasubramanian
HVP1214	*h^{90} map4::ura4^{+} leu1-32 ade6*	Lab stock
HVP1236	*h^{90} sec8-1 leu1-32 ura4-D18 ade6*	Lab stock
HVP1237	*h^{90} exo70::ura4^{+} leu1-32 ade6*	Lab stock
HVP1350	*h^{90} map4::ura4^{+} map4-GFP:leu1^{+} ade6*	Lab stock
HVP1428	*h^{90} map4-HA:leu1^{+} ura4-D18 ade6*	Lab stock
HVP1443	*h$^{?}$ spm1::LEU2 ura4-D18*	J.A. Cooper
HVP1587	*h^{90} spm1::LEU2 ura4-D18*	This work
HVP1659	*h^{90} cfr1::his3^{+} map4-GFP:KAN leu1-32 ura4-D18 ade6*	This work
HVP1660	*h^{90} sec8-1 map4-GFP:KAN leu1-32 ura4-D18 ade6*	This work
HVP1661	*h^{90} exo70::ura4^{+} map4-GFP:KAN leu1-32 ade6*	This work
HVP1662	*h^{90} spm1::LEU2 map4-GFP:KAN ura4-D18*	This work
HVP1665	*h^{90} dni1::KAN map4-GFP:leu1^{+} ura4-D18 ade6*	This work
HVP1949	*h^{90} sec8-1 map4-HA:leu1^{+} ura4-D18 ade6*	This work
HVP1952	*h^{90} spk1::ura4^{+} leu1-32 ade6*	M. Yamamoto
HVP1968	*h^{90} spk1::ura4^{+} map4-GFP:leu1^{+} ade6*	This work
HVP1969	*h^{90} spk1::ura4^{+} map4-HA:leu1^{+} ade6*	This work
HVP1997	*h^{90} exo70::ura4^{+} map4-HA:leu1^{+} ade6*	This work
HVP2032	*h^{90} GFP-Psy1:leu1^{+}*	YGRC (FY12296)
HVP2033	*h^{90} meu14-GFP*	YGRC (FY12492)
HVP2036	*h^{-} mok12-GFP:KAN leu1-32 ura4-D18*	Y. Sanchez
HVP2037	*h^{-} mok13-GFP:KAN leu1-32 ade6*	Y. Sanchez
HVP2102	*h^{90} exo70::ura4^{+} mok12-GFP:KAN leu1-32*	This work
HVP2103	*h^{90} exo70::ura4^{+} mok13-GFP:KAN leu1-32 ade6*	This work
HVP2104	*h^{90} exo70::ura4^{+} GFP-Psy1:leu1^{+}*	This work
HVP2119	*h^{90} exo70::ura4^{+} meu14-GFP*	This work
HVP2154	*h^{-} sec8-GFP:ura4^{+} leu1-32*	M. Balasubramanian
HVP2173	*h^{90} mok12-GFP:KAN leu1-32 ura4-D18*	This work
HVP2174	*h^{90} mok13-GFP:KAN leu1-32 ura4-D18*	This work
HVP2175	*h^{90} sec8-GFP:ura4^{+} leu1-32 ade6?*	This work
HVP2190	*h^{90} exo70-RFP:KAN leu1-32 ade6 ura4D-18*	This work
HVP2263	*h^{90} sec8-GFP:ura4^{+} exo70-RFP:KAN leu1-32 ade6?*	This work
HVP2272	*h^{90} sec8-1 GFP-Psy1:leu1^{+} ura4-D18 ade6?*	This work
HVP2274	*h^{90} sec8-1 mok12-GFP:KAN ura4-D18 leu1-32 ade6?*	This work

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