Identification of Genes Controlling Growth Polarity in the Budding Yeast *Saccharomyces cerevisiae*: A Possible Role of *N*-Glycosylation and Involvement of the Exocyst Complex

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ABSTRACT

The regulation of secretion polarity and cell surface growth during the cell cycle is critical for proper morphogenesis and viability of *Saccharomyces cerevisiae*. A shift from isotropic cell surface growth to polarized growth is necessary for bud emergence and a repolarization of secretion to the bud neck is necessary for cell separation. Although alterations in the actin cytoskeleton have been implicated in these changes in secretion polarity, clearly other cellular systems involved in secretion are likely to be targets of cell cycle regulation. To investigate mechanisms coupling cell cycle progression to changes in secretion polarity in parallel with and downstream of regulation of actin polarization, we implemented a screen for mutants defective specifically in polarized growth but with normal actin cytoskeleton structure. These mutants fell into three classes: those partially defective in *N*-glycosylation, those linked to specific defects in the exocyst, and a third class neither defective in glycosylation nor linked to the exocyst. These results raise the possibility that changes in *N*-linked glycosylation may be involved in a signal linking cell cycle progression and secretion polarity and that the exocyst may have regulatory functions in coupling the secretory machinery to the polarized actin cytoskeleton.

THE budding yeast *Saccharomyces cerevisiae* undergoes successive phases of isotropic and polarized growth during the cell cycle. After commitment to a round of division, growth must be polarized to generate a recipient bud that will receive the daughter nucleus during mitosis (LEW and REED 1995b). In early G1 phase, isotropic growth allows cells to reach a critical size for entering into the mitotic cell cycle. After execution of START, the regulatory event that commits cells to a division cycle, growth is polarized to the presumptive bud site and then to the emerging bud. This apical growth pattern continues early during the budded phase, partially depolarizes during the late budded phase and becomes isotropic again transiently during mitosis. Secretion is finally redirected to the bud neck at the end of mitosis, presumably to provide activities necessary for separation of mother and daughter cells (BYERS 1981). A consequence of this highly regulated sequence of cell surface growth patterns is the ellipsoidal shape characteristic of wild-type yeast.

Early studies emphasized the role of the actin cytoskeleton in mediating polarized growth (ADAMS and PRINGLE 1984; KILMARTIN and ADAMS 1984). A strong correlation between actin localization and polarized growth at specific sites of the cell surface led to the idea that secretory vesicles are directed toward these sites via actin cables and cortical actin patches. The observation that mutations in the actin gene, genes encoding actin binding proteins or genes involved in actin polarization affect both polarized growth and bud emergence was also consistent with the idea that the actin cytoskeleton plays an important role in directing secretion to specific regions of the cell surface (WELCH *et al.* 1994; DRUBIN and NELSON 1996). The mechanisms governing this process are not well characterized although electron microscopic studies suggested that actin patches are associated with the cell surface through an invagination of the plasma membrane (MULHOLLAND *et al.* 1994). Furthermore, it was observed that the secretory machinery itself, endoplasmic reticulum and Golgi apparatus, is polarized early during bud emergence (PREUSS *et al.* 1992), but a physical interaction between these secretory organelles and the actin cytoskeleton has yet to be established.

Cell cycle progression and the morphogenesis (budding) cycle in yeast are tightly coupled as evidenced by the concomitance of bud emergence and S phase initiation under normal conditions (REED 1992). The successive rearrangements of the actin cytoskeleton have been shown to be under the control of the cell cycle machinery itself (LEW and REED 1993). Activation of Cdc28p by the G1 cyclins (Clnp) at START triggers actin polarization to the presumptive bud site. The switch to less polarized growth in the bud is later mediated by activation of Cdc28p by the G2/M cyclins

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(Clbp) and inactivation of Clnp/Cdc28p complexes. Finally, inactivation of Cdc28p at the end of mitosis allows secretion to be repolarized to the bud neck for cytokinesis and cell separation to proceed. The essential coordination between cell cycle progression and the budding cycle is ultimately maintained by the presence of a checkpoint: in response to a delay or block of bud emergence, nuclear division is delayed to provide time for cells to eventually produce a bud, thus avoiding the generation of multinucleated cells (LEW and REED 1995a,b). This delay was recently shown to be mediated primarily through activation of Swe1p, a protein kinase that negatively regulates Cdc28p by phosphorylation on tyrosine 19 (SIA *et al.* 1996).

Although remodeling the actin cytoskeleton is necessary for alterations of polarized growth at the different phases of the budding cycle, it does not seem to be sufficient. A mutation designated *bed1-1* was shown to specifically impair polarized growth and bud emergence without ostensibly interfering with the structure of the actin cytoskeleton (MONDÉSERT and REED 1996). In *bed1* cells, bud emergence is delayed by more than one hour although actin polarization and S phase proceed on a normal schedule. These cells can survive such morphogenetic defects only if the morphogenesis checkpoint machinery is fully operative, as demonstrated by the fact that they die as multinucleated cells when the checkpoint is overridden by overexpression of the mitotic cyclin Clb2p (MONDÉSERT and REED 1996). To investigate the mechanisms that control growth polarity downstream of or in parallel with the actin cytoskeleton, we identified and characterized mutants defective in growth that maintained normal actin organization. The characterization of these mutants and the genes defined by them is the subject of this report.

MATERIALS AND METHODS

Yeast strains, media and growth conditions: All strains used in this study were derivatives of BF264-15DU: *MATa ade1 his2 leu2-3,112 trp1-1^a ura3Dns* (RICHARDSON *et al.* 1989). The relevant genotypes of strains used in this study are shown in Table 1. Yeast cultures were grown at 30° unless specified in YEP medium containing 2% dextrose (YEPD), raffinose (YEPR) or galactose (YEPG) as a carbon source. Induction of the *GAL1:CLB2* allele was achieved by addition of 2% galactose to a mid-log phase culture (YEPR) for 4–8 hr.

Isolation of *mcd* mutant strains: The *mcd* mutants (morphogenesis checkpoint dependent) described in this article were isolated through essentially the same protocol that led to the isolation of the *bed1-1* mutation and the identification of the *BED1/MNN10* gene (Figure 1). A strain carrying an inducible *GAL1:CLB2* allele (GY-1007) was mutagenized with ultraviolet radiation (70% death) on YEPD plates (*GAL1* promoter repressed) and incubated at 25° for 2 days. The colonies were replica-plated to YEPG plates (*GAL1* promoter induced) at 37° and candidates that did not grow were then retested for temperature sensitivity on YEPD and YEPG leading to the isolation of 80 mutant strains that were inviable on galactose medium or more temperature sensitive on galactose than on dextrose medium. These candidates were then analyzed for DNA content by FACS analysis, for the number of nuclei per cell after overexpression of Clb2p in YEPG and for morphology in YEPD at the restrictive temperature. Three categories were established according to these criteria (see Figure 1): mutants presumably unable to utilize galactose as a carbon source (no specific cell cycle arrest and no morphogenetic defects), cell cycle mutants unable to tolerate high levels of Clb2p (specific cell cycle arrest and no morphogenetic defects) and morphogenetic mutants unable to survive when the morphogenesis checkpoint is completely overridden by Clb2p overexpression (multinucleated cells with morphogenetic defects). This latter category (*mcd* mutants) was analyzed for the status of the actin cytoskeleton and 11 mutants without any obvious defect in actin polarization were selected for further study.

Each of these 11 *mcd* mutants was then backcrossed to the wild-type strain GY-1002 to determine whether the mutations were recessive. Tetrad analysis of these diploid strains showed that the lethality was associated with overexpression of Clb2p, *i.e.*, that the lethality on galactose medium was not observed without the *GAL1:CLB2* allele, and that this lethality was conferred by a single mutation unlinked to the *GAL1:CLB2* locus. The 11 *mcd* mutants, including the *bed1-1* mutation previously identified, were assigned to seven different complementation groups (see Table 3 for a summary).

Identification and molecular characterization of the *MCD* genes: The *MCD* genes were cloned by complementation of the lethality induced by overexpression of Clb2p. A YCp50-based yeast genomic DNA library was introduced into mutant strains by transformation (ROSE *et al.* 1987). The screening of between 10,000 and 20,000 transformants yielded one or two plasmids for each mutant. Sequencing of the 5′ and 3′ extremities of the inserts contained in these plasmids allowed us to locate the relevant chromosomal segment in the genome. Subsequent subcloning led to the identification of candidate genes corresponding to the different *mcd* mutations (for a summary, see Table 3).

Putative *MCD* loci on plasmids were marked with the *URA3* gene (or with the kanamycin resistance gene for the *MCD7* locus) and integrated by homologous recombination to confirm that the *MCD* genes cloned by complementation indeed corresponded to the *MCD* loci defined mutationally. Between 25 and 40 tetrads from *mcd/MCD* (*URA3/KAN^R*) diploid strains were analyzed and the genetic distance between the mutation and the corresponding marked gene was determined (Table 3). We demonstrated that the *mcd6-82* mutation is genetically linked to the *PRE1* locus: the distance between the *mcd6-82* and *pre1-1* mutations was evaluated to be roughly 10 cM, in good agreement to the physical distance between the two loci (~10 kb).

Strain construction: Strains were constructed according to standard genetic procedures (SHERMAN *et al.* 1982) except that gene disruptions using the kanamycin-resistance gene (*KAN^R*) were performed as previously described (WACH *et al.* 1994).

The Sec5 protein was tagged with three HA epitopes at the C-terminal extremity of the genomic coding region using the pKHA3 (*KAN^R*) vector (S. B. HAASE, M. WOLFF and S. I. REED, unpublished results). Briefly, the C-terminal portion of the *SEC5* open reading frame (ORF) (chromosome IV, 787,294–786,347) was amplified by PCR using primers containing a 5′ *Sal*I site and a 3′ *Not*I site (primers: 5′-**GTCGAC**CAACTG GAGAACTGGCAGGTCTAT-3′ and 5′-**GCGGCCGC**GCT GAAGGCGGCGAATTGAATAGC-3′; the *Sal*I and *Not*I sites are indicated in bold characters). The PCR product was then sequenced, to verify that no mutation had been introduced during the procedure, and cloned into the pKHA3 vector in

TABLE 1

***S. cerevisiae* strains used in this study**

Strain	Genotype
GY-381	*MATa BAR1 bed1::URA3*
GY-382	*MATa BAR1 bed1::URA3 GAL1:CLB2(LEU2)*
GY-1001	*MATa barlΔ ade1 his2 leu2-3,112 trp1-1a ura3Dns ARG4*
GY-1002	*MATα BAR1 ade1 his2 leu2-3,112 trp1-1a ura3Dns-ARG4*
GY-1007	*MATa BAR1 GAL1:CLB2(LEU2) arg4*
GY-1015	*MATa/MATα GAL1:CLB2(LEU2)/leu2 arg4/ARG4*
GY-1017	*MATα BAR1 GAL1:CLB2(LEU2) arg4*
GY-1116	*MATa BAR1 sec5-19 GAL1:CLB2(LEU2) arg4*
GY-1087	*MATa BAR1 sec53-71 GAL1:CLB2(LEU2) arg4*
GY-1341	*MATa BAR1 sec3-82 GAL1:CLB2(LEU2) arg4*
GY-1342	*MATa BAR1 sec53-112 GAL1:CLB2(LEU2) arg4*
GY-1343	*MATa BAR1 anp1-139 GAL1:CLB2(LEU2) arg4*
GY-1344	*MATa BAR1 sec53-141 GAL1:CLB2(LEU2) arg4*
GY-1125	*MATa BAR1 mcd4-154 GAL1:CLB2(LEU2) arg4*
GY-1127	*MATa BAR1 vrg4-160 GAL1:CLB2(LEU2) arg4*
GY-1136	*MATa BAR1 mcd4-174 GAL1:CLB2(LEU2) arg4*
GY-1142	*MATa BAR1 anp1-186 GAL1:CLB2(LEU2) arg4*
GY-1155	*MATa/MATα MNN9/mnn9::KAN^R leu2/GAL1:CLB2(LEU2) arg4/ARG4*
GY-1158	*MATa mnn9::KAN^R ARG4*
GY-1160	*MATa mnn9::KAN^R GAL1:CLB2(LEU2) arg4*
GY-1164	*MATa/MAT α OCH1/och1::KAN^R leu2/GAL1:CLB2(LEU2) arg4/ARG4*
GY-1166	*MATa och1::KAN^R ARG4*
GY-1168	*MATa och1::KAN^R GAL1:CLB2(LEU2) arg4*
GY-1191	*MATa barΔ SEC5[HA]3X(KAN^R) ARG4*
GY-1192	*MATα barlΔ SEC5[HA]3x(KAN^R) ARG4*
GY-1261	*MATa* pCYI20(*URA3*) *ARG4*
GY-1262	*MATamnn9::KAN^R GAL1:CLB2(LEU2)* pCYI20(*URA3*)
GY-1263	*MATa vrg4-160 GAL1:CLB2(LEU2)* pCYI20(*URA3*)
GY-1264	*MATa anp1-139 GAL1:CLB2(LEU2)* pCYI20(*URA3*)
GY-1266	*MATa sec53-71 GAL1:CLB2(LEU2)* pCYI20(*URA3*)
GY-1267	*MATa sec5-19 GAL1:CLB2(LEU2)* pCYI20(*URA3*)
GY-1268	*MATa mcd4-154 GAL1:CLB2(LEU2)* pCYI20(*URA3*)
GY-1329	*MATa barlΔ SEC53::SEC53(URA3)*
GY-1329	*MATa barlΔ MCD4::MCD4(URA3)*
GY-1330	*MATa barlΔ ANP1::ANP1(URA3)*
GY-1331	*MATa barlΔ VRG4::VRG4(URA3)*
PKY-129	*MATa pre1-1 arg4 leu2*
GY-1334	*MATa/MATα BAR1/barΔ anp1-186/ANP1::ANP1(URA3) GAL1:CLB2(LEU2)/leu2 arg4/ARG4*
GY-1335	*MATa/MATα BAR1/barlΔ sec53-71/SEC53::SEC53(URA3) GAL1:CLB2(LEU2)/leu2 arg4/ARG4*
GY-1336	*MATa/MATα BAR1/barlΔ mcd4-154/MCD4::MCD4(URA3)*
GY-1337	*MATa/MATα BAR1/barlΔ vrg4-160/VRG4::VRG4(URA3) GAL1:CLB2(LEU2)/leu2 arg4/ARG4*
GY-1338	*MATa/MATα barlΔ/barlΔ sec5-19/SEC5[HA]3X(KAN^R) GAL1:CLB2(LEU2)/leu2 arg4/ARG4*
GY-1339	*MATa/MATα BAR1/BAR1 sec3-82/SEC3 PRE1/pre1-1 GAL1:CLB2(LEU2)/leu2 arg4/arg4*

frame with a triple HA epitope followed by a STOP codon and a transcription terminator. This plasmid (pKHA3:SEC5) was linearized with *Bgl*II and transformed into yeast. The tagged strains (GY-1191 and GY-1192) were wild type by all the criteria that were tested, suggesting that the tagged protein was fully active. We could detect the tagged Sec5 protein as a single band migrating at 105 kD, in good agreement with the predicted molecular weight.

Cell biology protocols: FACS analysis was performed as previously described (LEW *et al.* 1992). Nuclei were visualized either using cells stained with propidium iodide or by DAPI staining (MONDÉSERT and REED 1996). Actin and chitin staining and immunolocalization of the tagged Sec5 protein was performed as previously described (PRINGLE 1991; PRINGLE *et al.* 1991; MONDÉSERT and REED 1996).

***In situ* invertase gel assay:** Native gel electrophoresis and in gel assay were performed essentially as previously described (BALLOU *et al.* 1991; POSTER and DEAN 1996). Briefly, cells carrying a plasmid overexpressing invertase were grown 1° below the restrictive temperature in dextrose medium to mid-log phase. Approximately 10^7 cells were harvested, washed in TP buffer (10 mM Tris-HCl pH 7.5, 1 mM PMSF) and broken with glass beads in 50 ml TP buffer at 4°. The same volume of TP buffer containing 20% glycerol and 0.01% bromophenol blue was added and samples were clarified by centrifugation and loaded on a 6% native acrylamide gel. In gel activity assays were performed as previously described (BALLOU *et al.* 1991).

We were not able to detect invertase activity in our genetic background (BF264-15DU), suggesting the strains used in this study contained very low amounts of, if any, invertase activity, in good agreement with the fact that 15D strains grow poorly

in filtered sucrose medium. Therefore, we transformed the strains to be analyzed with the pCY20 plasmid that allows constitutive overexpression of invertase [*SUC2* coding region fused to the *CPY* promoter and its signal sequence coding region (JOHNSON *et al.* 1987)].

RESULTS

In the interest of identifying components of the secretory pathway that control growth polarity during the cell cycle of budding yeast, we took advantage of the observation that cells severely defective in polarized growth depend for survival on the morphogenesis checkpoint, which delays mitosis in cells that have not budded. Mutations were identified that conferred conditional lethality, when the morphogenesis checkpoint was overridden. This approach carried out on a small scale led initially to the identification of Bed1p/Mnn10p, a membrane protein required for the establishment of growth polarity at the G1/S transition (MONDÉSERT and REED 1996). We extended this screen and characterized a total of 11 mutations, defining seven gene products implicated directly or indirectly in growth polarity but not in actin structure or polarization.

Identification of *mcd* mutations: A strain overexpressing the mitotic cyclin Clb2p under the control of the inducible *GAL1* promoter is not only delayed for the exit from mitosis but is also incapable of cell cycle delay in response to the morphogenesis checkpoint, under conditions where bud emergence is impaired (LEW and REED 1995a). Such cells were mutagenized by UV irradiation and mutant strains that were not viable on galactose medium or more temperature sensitive on galactose medium (*GAL1:CLB2* induced) than on dextrose medium (*GAL1:CLB2* repressed) were selected (for a more detailed description of this screen, see MATERIALS AND METHODS). The rationale for this approach was that mutations impairing polarized growth should be sensitive to override of the morphogenesis checkpoint. The incorporation of a high temperature shift into the screen was to allow for the isolation of temperature sensitive mutations, should targeted genes be essential. A combination of DNA content analysis and microscopic observation lead to the identification of three categories of mutations from the analysis of a total of 100,000 mutagenized colonies (Figure 1): first, mutations presumably affecting the ability of the cells to proliferate when using galactose as a carbon source (no cell cycle defects and no morphogenetic aberrations: 37 mutant strains); second, mutations involved in cell cycle regulation leading to the inability to tolerate elevated levels of Clb2p (specific cell cycle arrest and no morphogenetic aberrations: two mutant strains); third, mutations impairing polarized growth and/or bud emergence (multinucleated cells and morphogenetic defects: 19 mutant strains). The analysis of this last category (*mcd* mutations, for morphogenesis checkpoint dependent) is described in this report.

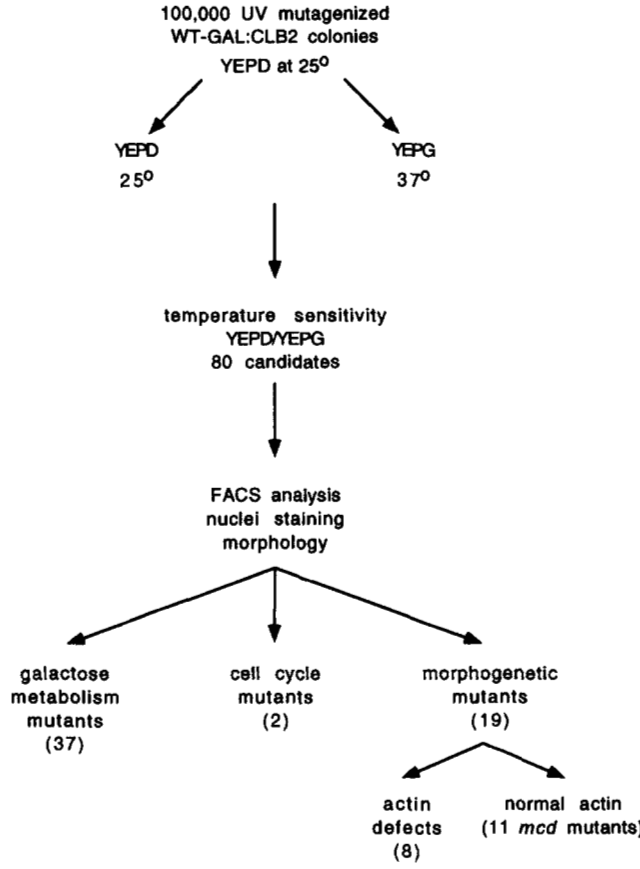

FIGURE 1.—Schematic summary of the screen used to isolate *mcd* mutants.

Most of the morphogenetic mutations described (mutations affecting bud emergence) in the literature are associated with defects in the establishment or maintenance of actin polarization toward sites of active growth (presumptive bud site at the G1/S transition, tip of the bud in S phase and bud neck during cytokinesis and cell separation). Among 19 morphogenetic mutant strains identified in this screen, eight showed such defects and a more detailed analysis of two of these led to the identification of new alleles of the previously described genes *VRP1* and *TPM1*, coding for proteins involved in actin organization [data not shown (LIU and BRETSCHER 1989; DONELLY *et al.* 1993)]. The remaining 11 mutant strains did not show any obvious defects in actin polarization at restrictive temperature and were therefore chosen for further study. Genetic analysis indicated that these mutant strains fall into seven complementation groups, including the previously described *BED1/MNN10* gene (see Table 3). Since only one allele was recovered for four of the complementation groups, the screen cannot be considered to be near saturation for *mcd* mutations.

Phenotypic analysis of the *mcd* mutants: Detailed analysis of the *mcd* mutants showed that *mcd2, 3* and *4*

were phenotypically similar to the *bed1::URA3* (*mcd1*) strain described previously (MONDÉSERT and REED 1996). The other mutants fell into classes, each with their own characteristic features. A detailed description of the phenotype of *mcd2, mcd5, mcd6* and *mcd7* mutants is therefore presented below.

The viability of mcd mutants depends on the integrity of the morphogenesis checkpoint: Wild-type cells overexpressing the mitotic cyclin Clb2p did not show any drastic cell cycle defect and the occurrence of multinucleated cells was very low (less than 1%), indicating that the synchrony between the mitotic cycle and the budding cycle is maintained even in the absence of a functional morphogenesis checkpoint. On the other hand, *mcd* mutants arrested with a large fraction of cells containing more than one nucleus when the morphogenesis checkpoint was abrogated by overexpression of Clb2p (see Figure 2). The percentage of multinucleated cells and the number of nuclei per cell varied for the different mutants, probably in proportion to the severity of the growth polarity defects of the different strains. For example, *mcd2* cells were nearly all multinucleated and cells with up to four nuclei could be detected. *mcd5* cells, on the other hand, rarely had more than two nuclei (Figure 2). Furthermore, in wild-type cells, nuclear division occurred normally when the recipient bud reached a significant and characteristic size. However, daughter nuclei could be observed in very small buds of *mcd* mutants with an intact morphogenesis checkpoint (Figure 2), suggesting that checkpoint-delayed nuclear division occurs very rapidly after bud emergence. This is evidence that the morphogenesis checkpoint plays an important role in the cell cycle dynamics of these mutants, delaying mitosis, but only until bud emergence has been initiated.

Determination of DNA content by FACS analysis showed that all but one of the *mcd* mutants were arrested with greater than 2N DNA content when Clb2p was overexpressed: all mutants but *mcd5* and *mcd6* arrested with most cells having a 4N DNA content (Figure 2). FACS analysis of *mcd5* mutants showed, on the other hand, populations having 2N and 3N DNA contents, in good agreement with the observation, based on microscopic analysis, of unbudded cells containing two nuclei and cells with an unseparated daughter, where the mother cell contains two nuclei and the daughter cell contains one nucleus. The FACS profile of *mcd6* cells showed a majority with a 2N DNA content and a small fraction with a greater DNA content although a significant portion of these cells were multinucleated based on microscopic analysis (Figure 2).

In dextrose medium (*GAL1:CLB2* allele repressed), *mcd* mutants with more than one nucleus per cell were rarely detected under conditions of impaired polarized growth but where the morphogenesis checkpoint is operating normally (data not shown). Although FACS analysis showed that most *mcd2* cells had a 2N or greater DNA content (Figure 2), this distribution is most likely the result of defects in cell separation after cytokinesis observed in this strain (see below). *mcd5, mcd6* and *mcd7* mutants were temperature sensitive and arrested with a 2N DNA content at the restrictive temperature but with one nucleus per cell. The fact that *mcd* cells in dextrose medium rarely have more than one nucleus per cell suggests that the morphogenesis checkpoint control machinery is functional in these cells at temperatures where defects in polarized growth occur. Taken together, these results suggest that the lethality of *mcd* mutants in galactose medium is due to the abrogation of the morphogenesis checkpoint when Clb2p is overexpressed and that the viability of these cells depends on the integrity of this checkpoint.

Morphogenetic defects of the mcd mutants: Microscopic evaluation of the morphology of the *mcd2, 3, 4, 6* and *7* strains revealed three types of morphogenetic defect (Figure 3): these cells had (1) lost the ellipsoidal shape characteristic of wild-type cells and were abnormally round, (2) were larger than wild-type cells and (3) had a cell separation and aggregation phenotype. The first two defects suggest that these cells are impaired in establishing polarized growth but that secretion itself is not affected. In contrast, *mcd5* mutants exhibited a wild-type ellipsoidal shape (Figure 3) and were only slightly larger than wild-type cells even after long incubations at the restrictive temperature. This suggests that growth polarity is not affected *per se* in these mutants but that secretion might be impaired in a more general sense, as previously described for *sec* mutants.

The growth polarity defects and the aggregation phenotype were more precisely assessed by determining the budding index before and after zymolase treatment (see Table 2). After only a mild sonication treatment, the budding index of the *mcd* mutant cells was similar to, if not higher than, that associated with wild-type cells. On the other hand, the budding index of *mcd* mutant cells was substantially decreased after zymolase treatment to levels significantly lower than wild-type cells. These data suggest that *mcd* mutants have bud emergence defects, in good agreement with the growth polarity phenotype and that the aggregation phenotype is probably due to cell separation defects rather than cytokinesis defects.

Staining cells with calcofluor, which detects chitin, was used as an indirect assay for scoring growth polarity in *mcd* mutants. Chitin is normally found concentrated in the neck region of budded cells and in bud scars of cells that have already produced a bud (Figure 4). Delocalization of chitin is often associated with a defect in polarized growth. Calcofluor-stained *mcd2, 6* and *7* cells showed delocalized and elevated levels of chitin (the staining was less intense in *mcd7* cells, see Figure 4) consistent with a defect in growth polarity. The staining pattern of *mcd5* cells showed that chitin deposition was only slightly delocalized in comparison with wild-type

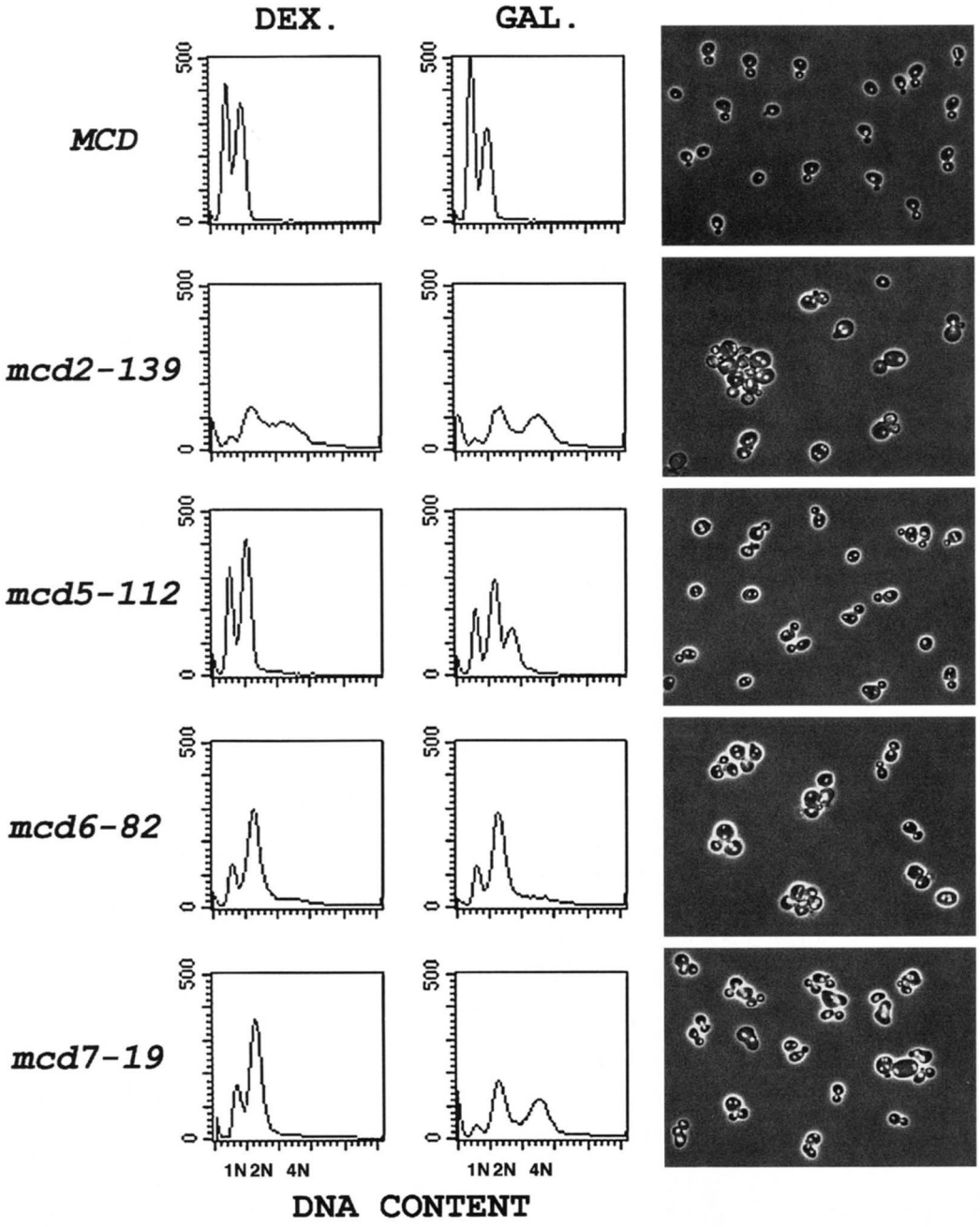

FIGURE 2.—The viability of *mcd* mutant cells depends on the integrity of the morphogenesis checkpoint. Wild-type and mutant cells carrying a *GAL1:CLB2* allele were grown to mid-log phase in YEPR at 25° and transferred to YEPD and YEPG medium at restrictive temperature (see Table 3) for 4 hr. Aliquots were taken for FACS analysis and the same cells were used for microscopic analysis to detect nuclei in the absence of a functional morphogenesis checkpoint (YEPG medium, *GAL1:CLB2* allele induced). Micrographs were generated by a combination of phase-contrast and fluorescence detection of DNA stained with propidium iodide.

cells, again arguing for more general or severe secretion defects in this mutant.

As mentioned above, actin goes through characteristic phases of polarization and depolarization as a function of progression through the cell cycle. These changes in actin structure appear to be necessary for establishing growth polarity. We focused our work on the mechanisms that govern growth polarity downstream or parallel to actin polarization. The *mcd* mutants described here did not exhibit any obvious defects in actin polarization at restrictive temperature in dextrose medium (Figure 4) as observed by staining with rhodamine-phalloidin. Cortical actin patches were concentrated in small buds presumably undergoing polarized growth, actin rings were detected at presumptive pre-bud sites and actin filaments were polarized to the sites of growth. We did not detect any strong defect in the structure of the actin cytoskeleton as in *vrp1* or *tpm1* mutant strains that we also recovered in this screen (not shown). However, it should be noted that actin became significantly depolarized only after long incubation periods at the restrictive temperatures of the *ts* mutants, in particular *mcd2, mcd5* and *mcd7*. This suggests that *MCD* genes are not directly involved in actin regulation but that chronic loss of Mcdp functions can induce structural changes of the actin cytoskeleton as was recently shown for *sec3* mutations (FINGER and NOVICK 1997).

In summary, with the exception of *mcd5,* which exhibited a more general defect in growth, *mcd* mutants were

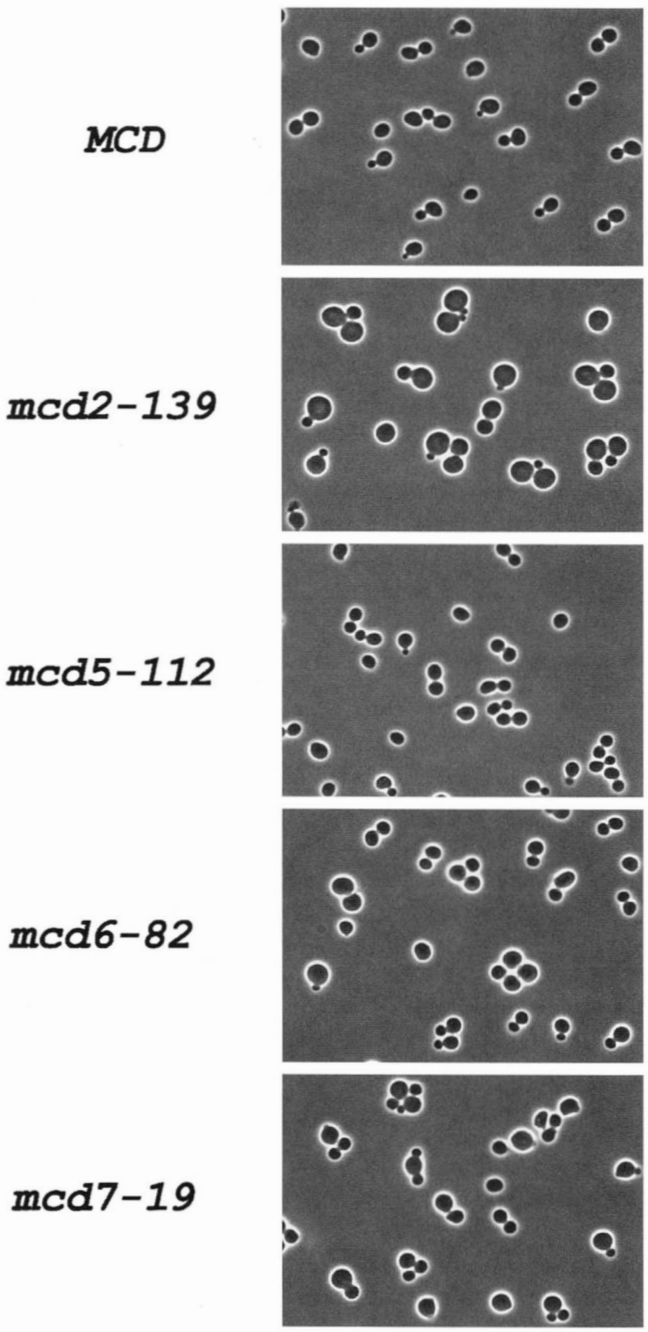

FIGURE 3.—Morphology of *mcd* mutant cells at the restrictive temperature in the presence of a functional morphogenesis checkpoint. Phase-contrast micrographs of wild-type and mutant cells that were grown to mid-log phase in YEPR at 25° and transferred in YEPD medium at restrictive temperature (see Table 3) for 4 hr. Magnification is the same for all strains.

affected specifically in the establishment of growth polarity while actin polarization was not disturbed in any obvious way.

Molecular identification of the *MCD* genes: The genes mutated in the *mcd* strains were cloned by rescue of the lethality resulting from the overexpression of Clb2p using a library of large genomic DNA fragments carried on a centromeric plasmid. Determination of the junction sequences of the inserts contained in the rescuing plasmids allowed us to use the yeast genome database to rapidly determine the genomic segments containing the *MCD* genes. Subsequent subcloning analysis of these large fragments allowed us to identify the *MCD* genes themselves (see Table 3 and MATERIALS AND METHODS). Genetic linkage analysis was performed to show that the cloned *MCD* genes indeed corresponded to the loci defined mutationally (see Table 3 and MATERIALS AND METHODS).

Some mcd mutations affect outer chain elongation of N-linked oligosaccharides: The lethality resulting from Clb2p overexpression in *mcd2* and *mcd3* backgrounds could be rescued with plasmids containing the *ANP1/GEM3* and *VRG4/VAN2* genes, respectively (see Table 3). *ANP1* was first identified as a gene involved in sensitivity to aminonitrophenyl propanediol and hypo-osmotic medium (MCKNIGHT *et al.* 1981). It was later identified as a gene involved in the maintenance of early Golgi localization of Mnt1p, an α-1,2-mannosyltransferase involved in both *O*-glycosylation and *N*-glycosylation (CHAPMAN and MUNRO 1994). Anp1p is part of a family of yeast type II integral membrane proteins that includes the closely related Van1p and the more distantly related Mnn9p. *VRG4/VAN2* was identified as a vanadate resistance gene and encodes an integral membrane protein that is required for normal Golgi function (KANIK-ENNULAT *et al* 1995; POSTER and DEAN 1996). The vanadate resistance phenotype is poorly understood but it was used as tool to enrich mutants defective in Golgi-specific glycosylation (BALLOU *et al.* 1991).

The identification of *ANP1* and *VRG4* in a screen that also led to the identification of *BED1/MNN10* was striking since several phenotypic aspects of mutations in these genes are similar: *anp1, vrg4* and *bed1/mnn10* strains are vanadate resistant, they have similar morphologies and defects in establishing growth polarity and they have defects in glycosylation (BALLOU *et al.* 1991; KANIK-ENNULAT *et al* 1995; DEAN and POSTER 1996; POSTER and DEAN 1996). This prompted us to determine if this latter phenotype was common to all *mcd* strains. As shown in Figure 5, *mcd2* and *3* mutants showed various degrees of defective glycosylation when tested by evaluation of the degree of invertase modification. Cells disrupted in the *BED1/MNN10* gene (*mcd1* complementation group) showed intermediate defects similar to those of *mcd2* mutants (not shown). Based on previous studies on some of these genes, this impairment of invertase modification is probably indirect and due to defects in Golgi organization, at least in *anp1* and *vrg4* mutant cells (BALLOU *et al.* 1991; CHAPMAN and MUNRO 1994; KANIK-ENULAT *et al.* 1995; POSTER and DEAN 1996).

mcd5 mutant cells also exhibited a strong defect in invertase glycosylation (Figure 5). Since *mcd5* mutations

TABLE 2

Bud emergence defects and aggregation phenotype of *mcd* mutants

					After zymolase treatment			
	No bud	1 bud	>1 bud	B.I. (%)	No bud	1 bud	>1 bud	B.I. (%)
WT	237	294	0	55	182	198	0	51
mcd2-139	75	97	50	66	135	69	5	35.5
mcd3-160	65	94	33	66	164	34	2	18
mcd4-154	103	145	25	62	224	65	2	23
mcd5-71	28	124	86	88	240	49	14	20.5
mcd6-82	152	144	20	52	159	60	5	32
mcd7-19	109	156	18	61.5	231	57	1	21
bed1Δ	101	135	14	59.5	168	68	14	32.5
mnn9Δ	24	84	122	89.5	121	67	31	44.5
och1Δ	45	125	175	87	178	94	34	42

Wild-type and mutant cells were grown to mid-log phase at 25° in YEPD medium and transferred at 37° for 5 hr or at 30° for *bed1Δ*, *mnn9Δ* and *och1Δ* cells. Budding index was determined after mild sonication and after zymolase treatment to evaluate the aggregation phenotype.

were mapped in the *SEC53* gene that encodes phosphomannomutase, these cells are likely to be defective in the production of GDP-mannose and therefore probably deficient in mannosyltransfer reactions at several steps of the secretory pathway.

We determined if other mutations directly or indirectly affecting glycosylation exhibited the same phenotypic characteristics as the *mcd* mutations. As mentioned above, the *MNN9* gene is structurally related to *ANP1; mnn9* strains are also vanadate resistant and have defects in outer chain elongation of *N*-linked oligosaccharides (BALLOU *et al.* 1991). *OCH1* also affects this process but in a more direct way, since it was shown to encode the α-1,6-mannosyltransferase that provides the initiating α-1,6-mannose on the Man8GlcNac[2] core oligosaccharide (NAKAYAMA *et al.* 1992). We disrupted these two nonessential genes in our strain background and demonstrated that invertase glycosylation defects are similar to those observed in the *mcd3-160* (*vrg4*) background (Figure 5). *mnn9Δ* and *och1Δ* strains are also defective in polarized growth, as judged by their morphology (Figure 6) and their budding index (Table 2), and their viability is dependent on the integrity of the morphogenesis checkpoint control machinery since overexpression of Clb2p was lethal, these cells becoming multinucleated as did other *mcd* mutants (Figure 6).

These results support the idea that outer chain elongation of *N*-linked oligosaccharides might be a signal involved in regulating polarized growth during the cell cycle (see DISCUSSION).

Identification of MCD4, a gene encoding a new membrane protein: The lethality of *mcd4* mutations after overexpression of Clb2p could be rescued by a DNA fragment carrying an ORF designated *YKL165c*. This gene encodes a probable integral membrane protein that is structurally conserved throughout evolution, as mammalian and invertebrate homologues were identified in database searches (unpublished results). Preliminary results suggest that the *MCD4* gene is essential for vegetative growth and encodes a protein localized to the endoplasmic reticulum (unpublished results). *mcd4* mutations confer a defect either downstream of or in a different pathway from glycosylation since the *mcd4-154* mutation did not affect the glycosylation status of invertase (Figure 5).

The late SEC genes, SEC3 and SEC5, components of the exocyst, are involved in the establishment of polarized growth: The lethality resulting from Clb2p overexpression in *mcd6* and *mcd7* backgrounds could be rescued by plasmids containing the *SEC3/PSL1* and *SEC5* genes, respectively. During the completion of this work, these "late" secretion defective (*SEC*) genes were identified as components of the "exocyst," a multiprotein complex involved in the regulation of exocytosis (TERBUSH *et al.* 1996). *SEC3* was also identified simultaneously through a screen for mutations synthetically lethal with a profilin mutation, suggesting that the exocyst might interact with the actin cytoskeleton to promote polarized growth (HAARER *et al.* 1996).

sec mutations were identified originally as completely defective in secretion (NOVICK *et al.* 1980). However, we did not find such severe defects associated with the alleles described in this article. The mutant cells were larger and rounder than wild-type cells (see Figure 3), two characteristics that argue against a complete block of the secretion pathway. Moreover, *mcd7-19* (an allele of *SEC5*) cells synchronized in G1 by centrifugal elutriation increased in size at the same rate as wild-type cells early in the cell cycle at restrictive temperature (not shown).

The localization of the Sec5 protein also argued for a role in polarized growth. This protein could be tagged at the C-terminal end with a triple HA epitope without

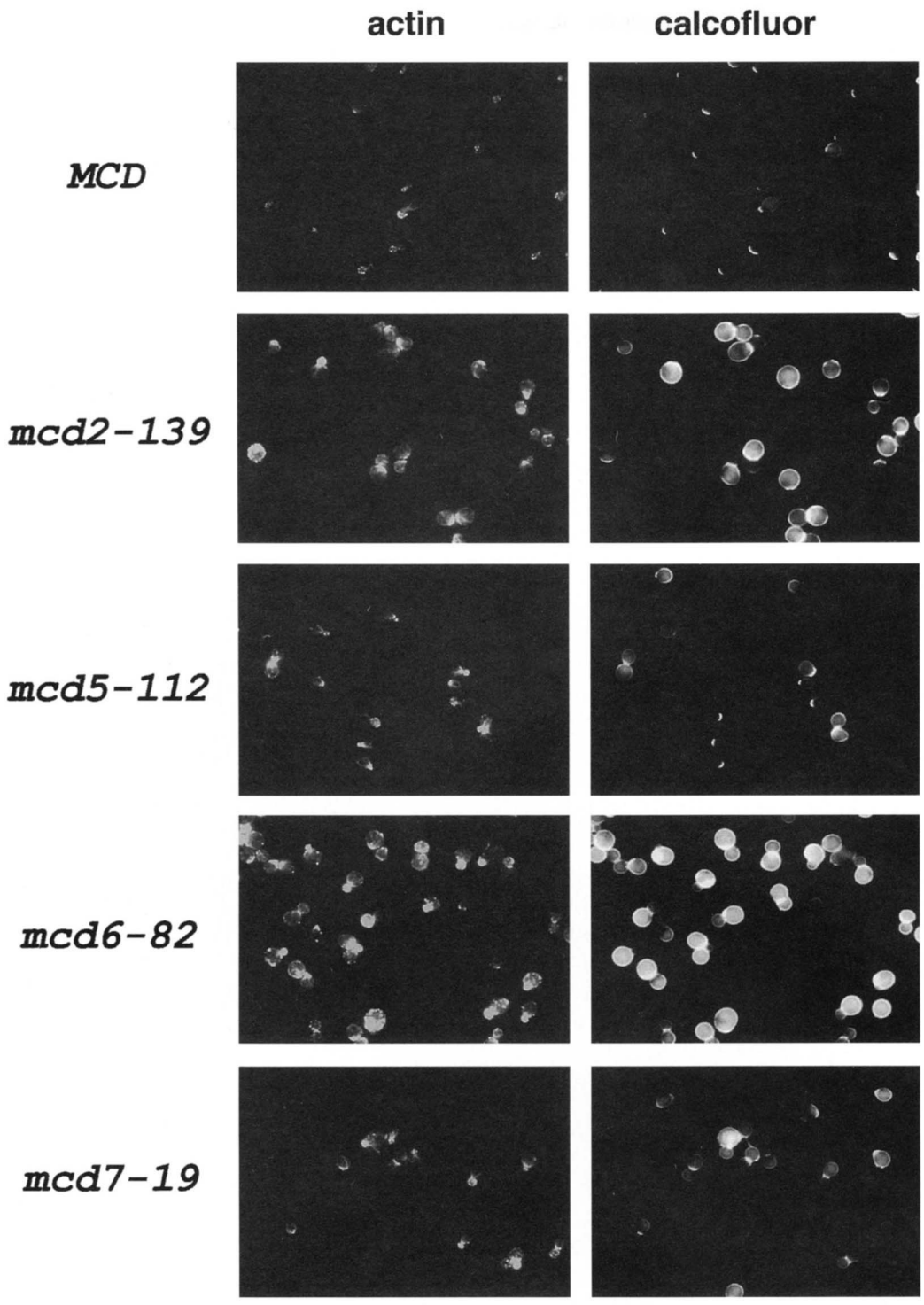

FIGURE 4.—Actin and chitin localization in *mcd* mutant cells. Wild-type and mutant cells were grown to mid-log phase in YEPR at 25° and transferred to YEPD or YEPG medium at the restrictive temperature (see Table 3) for 4 hr. Cells were fixed with formaldehyde and stained with rhodamine-phalloidin to visualize actin and with calcofluor to detect chitin. Magnification and exposure time are the same for all strains.

affecting its activity (see MATERIALS AND METHODS). Based on immunofluorescence microscopy, it was localized to sites where secretion is the most active and the most polarized, *i.e.*, at the pre-bud site, the tip of small buds and at the neck of large budded cells undergoing cytokinesis (Figure 7). Sec5p was also localized to the growing tip of cells responding to mating pheromone (shmoos), again consistent with a specific role in polarized secretion (Figure 7).

Altogether, these results suggest that the exocyst has a role in the polarization of secretion during the cell cycle (see below).

DISCUSSION

In *S. cerevisiae,* there is a critical interdependency of the budding cycle and the cell cycle clock. Cells defective in the process of establishing growth polarity and therefore forming a bud at the appropriate time in the cell cycle can survive only if the morphogenesis

TABLE 3

Genetic and molecular characterization of the different complementation groups obtained in this study

Group	Alleles	Restrictive temperature[a] DEX.	Restrictive temperature[a] GAL.	Minimal DNA fragment able to rescue	Gene	Genetic distance (cM)[b]
I[c]	*bed1-1*	+	−	Chromosome IV (952,194–954,785)	*BED1/MNN10*	<1
II	*mcd2-139*	+	−	Chromosome V (82,271–84,963)	*ANP1/GEM3*	<1
	mcd2-186	+	−			
III	*mcd3-160*	+	37°	Chromosome VII (76,405–78,636)	*VRG4/VAN2*	<1
IV	*mcd4-154*	35°	−	Chromosome XI (135,913–142,327	*MCD4/YKL165*[c]	<1
	mcd4-174	37°	35°			
V	*mcd5-71*	34°	30°	Chromosome VI (42,639–45,385[d])	*SEC53/ALG4*	<1
	mcd5-112	37°	35°			
	mcd5-141	+	34°			
VI	*mcd6-82*	34°	−	Chromosome V (167,620–172,047)	*PSL1/SEC3*	~10 (*pre1-1*)
VII	*mcd7-19*	37°	35°	Chromosome IV (786,344–789,259)	*SEC5*	*<1*

[a] The temperature sensitivity was tested at the following temperatures on YEPD and YEPG media: 25, 28, 30, 32, 34, 35, 36 and 37°; +, survive at all temperatures tested; −, did not survive at any temperature tested.

[b] The genetic distance was determined between the mutation and the *URA3* marker in the vicinity of the studied gene. In the case of *sec3-82,* the distance was determined between *sec3-82* and the *PRE1* locus (see MATERIALS AND METHODS).

[c] See MONDÉSERT and REED (1996).

[d] This DNA fragment also contains the *YFL046w* but this putative gene is not able to rescue the lethality on its own (see MATERIALS AND METHODS).

checkpoint control machinery is functional (LEW and REED 1995a). *bed1/mnn10* cells, which are defective in the most polarized form of secretion at the G1/S transition and during cytokinesis, cannot survive overexpression of the mitotic cyclin Clb2p because checkpoint-mediated G2 delay is completely abrogated under these circumstances (MONDÉSERT and REED 1996). We based the screen described in this article on these observations: by identifying mutant strains that were not able to tolerate elevated levels of Clb2p, we identified several genes that play a role in establishing growth polarity, independently of a role in controlling or maintaining the actin cytoskeleton. We assigned 11 different mutations to three groups, leading to a model implicating outer chain elongation of *N*-linked oligosaccharides as a potential signal for efficient polarized growth and suggesting that a multi-protein complex designated the exocyst might be part of the process linking the actin cytoskeleton and the secretory apparatus.

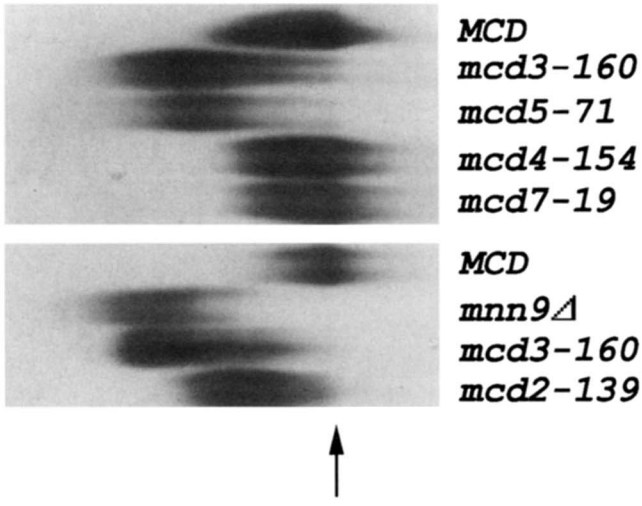

FIGURE 5.—Invertase modification is defective in some *mcd* mutants. Each mutant strain was grown in YEPD medium to mid-log phase at semi-permissive temperature where morphogenetic defects are apparent, *i.e.,* one degree below the restrictive temperature determined on plates (see Table 3, wild-type and *mnn9*Δ cells were grown at 30°). Extracts were loaded on a 6% native polyacrylamide gel and invertase was detected by in-gel activity staining. The position of fully glycosylated invertase is indicated with an arrow.

Mutants affected in *N*-linked glycosylation are defective in polarized growth: a signaling role for outer chain elongation of *N*-linked oligosaccharides? *mcd1, mcd2* and *mcd3* mutations were mapped to previously described genes involved directly or indirectly in *N*-linked glycosylation, namely, *BED1/MNN10, ANP1* and *VRG4. BED1,* which encodes a type II integral membrane protein demonstrated to be required for polarization of secretion at the G1/S transition and during cytokinesis, is identical to the *MNN10* gene (DEAN and POSTER 1996; MONDÉSERT and REED 1996). The *mnn10* mutation was originally isolated as conferring a defect in mannan structure (BALLOU *et al.* 1991). A strain deleted for this gene is viable but shows strong defects in bud emergence that are the direct consequence of impairment of polarized secretion. Bed1p/Mnn10p was shown to

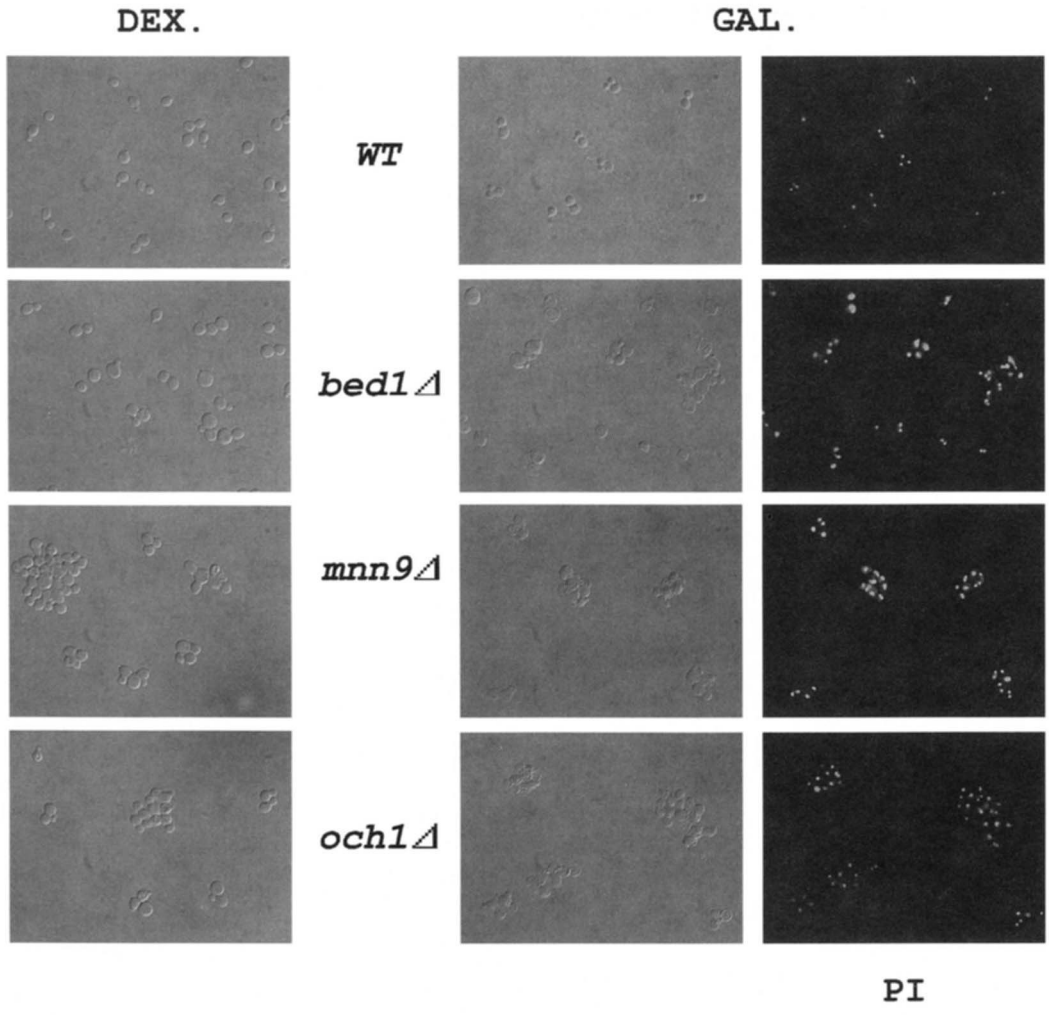

FIGURE 6.—Phenotypic analysis of *bed1*Δ, *mnn9*Δ and *och1*Δ cells. (A) Morphology of *bed1*Δ, *mnn9*Δ and *och1*Δ cells in the presence of a functional morphogenesis checkpoint. Normarsky micrographs of wild-type and mutant cells that were grown to mid-log phase in YEPR at 25° and transferred in YEPD medium for 4 hr. Magnification is the same for all strains. (B) The viability of *bed1*Δ, *mnn9*Δ and *och1*Δ mutant cells depends on the integrity of the morphogenesis checkpoint. Wild-type and mutant cells carrying a *GAL1:CLB2* allele were grown to mid-log phase in YEPR and transferred YEPG medium for 4 hr. Cells were processed as described in Figure 2.

belong to a family of related proteins, one of which is gma12p, an α-1,2-galactosyltransferase in *Schizosaccharomyces pombe* (CHAPPELL *et al.* 1994). However, Bed1p/Mnn10p is unlikely to be a galactosyltransferase since galactosyl modification of proteins does not occur in *S. cerevisiae*. The homology observed between Bed1p and gma12p could simply reflect the fact that these proteins interact with related polymannose structures or that Bed1p/Mnn10p possesses a different glycosyltransferase activity (DEAN and POSTER 1996; MONDÉSERT and REED 1996). Anp1p is also a type II integral membrane protein that was shown to be important for proper localization of modifying enzymes in the early Golgi pathway (CHAPMAN and MUNRO 1994). *VRG4* was cloned as a vanadate resistance gene and was shown to encode an integral membrane protein required for proper function of the Golgi apparatus (POSTER and DEAN 1996).

A common feature of these genes is that they are involved in *N*-linked glycosylation. Mutations affecting protein glycosylation have been classified in three groups according to the degree of severity of defects generated, as judged by the migration of invertase on nondenaturing gels. Glycosylation of invertase is most severely affected by the *vrg4* mutation in a manner similar to the *mnn9* mutation (Figure 5; see also POSTER and DEAN 1996). These defects were less severe with the *anp1* and *bed1/mnn10* mutations (Figure 5 and data not shown). Most of these mutations were shown to affect the maturation of *N*-linked oligosaccharides that occurs in the Golgi apparatus. We tested if mutations in other genes involved in this process were also defective in growth polarity in our strain background: *mnn9*Δ and *och1*Δ cells showed all the characteristics of the phenotype associated with *bed1/mnn10, anp1* and *vrg4* mutations. Another mutation showing defects in *N*-linked glycosylation was mapped to the *SEC53* gene that encodes for a phosphomannomutase, responsible for the synthesis of a precursor of mannosylation, GDP-mannose, necessary at several steps of the secretory pathway. Therefore, this mutation led to more severe defects in secretion (see below).

These results suggest that outer chain elongation of *N*-linked oligosaccharides might be part of a signaling pathway involved in polarization of secretion during the cell cycle. Several lines of evidence support this hypothesis. First, mutants affected in this process are viable and only polarized growth seems to be defective. Second, after treatment with tunicamycin, an inhibitor

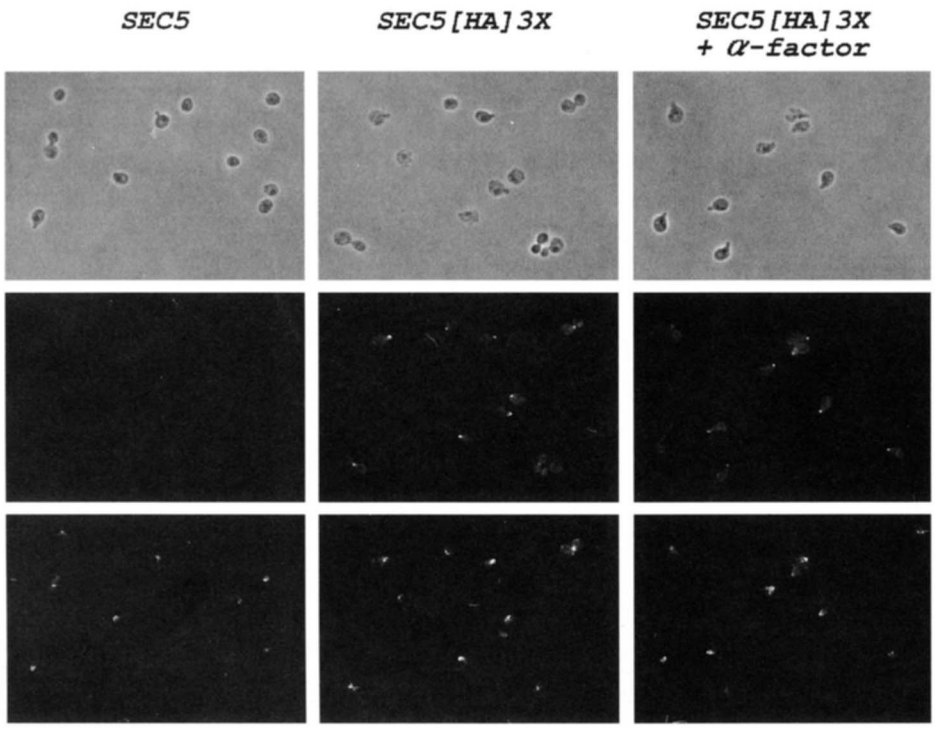

FIGURE 7.—Immmunolocalization of Sec5p. GY-1001 (wild-type strain, as a negative control) and GY-1191 (containing *SEC5[HA]3X*) cells grown in YEPD or in the presence of α-factor for 2 hr were stained with 12CA5 antibodies to detect the Sec5 fusion protein and at the same time with rhodamine-phalloidin to visualize actin. Magnification and exposure time are the same for both strains.

of *N*-linked glycosylation, yeast cells arrest as large unbudded cells with a 2N DNA content, an indication that only polarized growth is defective. The fact that cells arrest with a 2N DNA content is suggestive that the morphogenesis checkpoint was activated. Third, an analogous role has been proposed for *N*-glycans in protein sorting in epithelial cells, based on studies involving the effect of tunicamycin on the apical/basolateral routes from the Golgi apparatus to the plasma membrane (FIEDLER and SIMONS 1995). Moreover, apical secretion of rat growth hormone in epithelial cells was shown to be favored by addition of *N*-linked glycosylation sites (SCHEIFFELE *et al.* 1995). Finally, the genes coding for proteins involved in *N*-glycosylation contain SCB and MCB elements in their promoters, these elements being involved in cell cycle regulated transcription at the G1/S transition. The transcription of several of these genes was indeed shown to be regulated during the cell cycle (IGUAL *et al.* 1996). Taken together, these results raise the possibility that a boost in *N*-linked glycosylation is necessary not only to supply the increased need for mannoproteins in cell wall construction during bud emergence at the G1/S transition but also to direct secretion to the presumptive bud site and to the emerging bud. Alternatively, *N*-linked glycosylation could be rate limiting for the function of a protein specifically involved in polarized growth.

The model proposing that outer chain elongation of *N*-linked oligosaccharides provides a signal for sorting secretory vesicles from the Golgi apparatus to specific regions of the plasma membrane implies that this lumenal signal needs to be externalized to the vesicle surface allowing interaction with the actin cytoskeleton. Such a process could be fulfilled by one or several lectin-like molecule(s) that would recognize mature *N*-linked oligosaccharides and play the role of a "receptor." Thus, a signal necessary to target the vesicles to the most active growth sites via the actin cytoskeleton could be produced on the cytoplasmic surface. A similar model has been proposed for apical secretion in epithelial cells: lectin-like molecules, *ERGIC53* and *VIP36,* have been identified in these cells and appear to be involved in sorting proteins from the trans-Golgi network to the apical pole *vs.* the basolateral pole of these cells (FIEDLER and SIMONS 1995). Such lectin-like proteins have been detected in yeast: *EMP47* encodes a membrane protein that is localized at steady-state levels in the early Golgi but cycles to the endoplasmic reticulum (SCHRODER *et al.* 1995). The role of Emp47p is unknown, in that a deletion of *EMP47* shows no obvious growth defect. Since the yeast genome contains a gene highly homologous to *EMP47,* we tested the possibility that these two genes have redundant functions. However, the double deletion mutant showed no obvious growth defect (not shown). Therefore, lectin-like proteins specifically involved in establishing polarized growth in yeast remain to be identified.

Is the exocyst involved in polarized growth? Studies focused on the last step of the secretion pathway recently revealed the existence of a multiprotein complex, termed the exocyst, that is required for efficient exocytosis in *S. cerevisiae.* The exocyst consists of one molecule each of the proteins encoded by the late *sec* genes *SEC3, SEC5, SEC6, SEC8, SEC10, SEC15* and the novel gene *EXO70* (TERBUSH *et al.* 1996). Interestingly,

we recovered mutations in the *SEC3* and *SEC5* as preferentially affecting polarized growth.

We have shown that a tagged Sec5 protein was detected at sites where most polarized secretion takes place during the cell cycle, *i.e.*, the presumptive bud site at the G1/S transition, the tips of small buds in S phase, the necks of large budded cells late in cell cycle during cytokinesis and the growing tips of cells responding to mating pheromone (shmoos). This staining pattern is similar to that reported for Sec8p, another component of the exocyst, and for Sec4p, also involved in the regulation of exocytosis (NOVICK and BRENNWALD 1993; TERBUSCH and NOVICK 1995). These proteins were not reported to be localized at bud necks during cytokinesis probably due to detection sensitivity. The localization of the exocyst also argues for a specific role of this complex in polarized growth in addition to its more ubiquitous role in mediating exocytosis. Consistent with this, the *SEC3* gene was shown to be identical to *PSL1*, mutations in which are synthetically lethal with profilin mutations (HAARER *et al.* 1996; FINGER and NOVICK 1997). This suggests a link between exocytosis and polarization of growth through interaction with the actin cytoskeleton. In addition, the recent demonstration that proper localization of Sec4p and Sec8p requires actin function again emphasizes an active role of the exocyst complex in polarized growth (AYSCOUGH *et al.* 1997).

Analysis of the protein sequence of Sec5p revealed two nine-amino acid motifs [RLILQNNTN (positions 385–393) and RNTLSTIIN (positions 629–637)] similar to the destruction-box motif of B-type cyclins (RXALGXIXN; GLOTZER *et al.* 1991). We created deletion mutations in both motifs and showed that no dominant negative phenotype is detected when the mutant Sec5p is overexpressed under the *GAL1* promoter in an otherwise wild-type background (data not shown). Moreover, this mutated Sec5p is able to fully complement a deletion of the essential *SEC5* gene (data not shown). Consistent with these results, Sec5 protein levels do not fluctuate during the cell cycle (not shown). Therefore, these motifs are not critical for Sec5p function.

We isolated mutations in *SEC53*, a gene involved in secretion but probably at multiple stages of the pathway. *sec53* cells showed a more severe secretion phenotype than the late *sec* alleles that we isolated. *sec53* cells did not show an increase in cell size and arrested with different characteristics when the morphogenesis checkpoint was overridden: cells arrested primarily with two nuclei in the mother cell and only one in the daughter if present. This suggested that, in the absence of the checkpoint, nuclear division could occur once in the unbudded mother cell but not in the daughter cell, presumably due to growth constraints resulting from the severe secretion defect. It is unlikely that *sec53* mutants were sensitive to checkpoint override simply due to their severe secretion defect, as other similarly defective *sec* mutants were not isolated in the screen. Perhaps it is the defect in *N*-glycosylation exhibited by *sec53* mutants that sensitizes them, again consistent with a signaling role for glycosylation.

Polarized growth may be controlled at multiple levels: During recent years, a large number of studies on the control of growth polarity in yeast have suggested that this process is controlled at several levels. Early data demonstrating a strong correlation between actin polarity and polarity of secretion indicated a role of the actin cytoskeleton in polarizing growth to specific regions of the cell surface (WELCH *et al.* 1994). The polarity of the actin cytoskeleton was later shown to be regulated during the cell cycle by a succession of cyclin/Cdc28p complexes, the components of the cell cycle clock (LEW and REED 1993, 1995b).

The mechanisms by which the actin cytoskeleton controls growth polarity are poorly understood, although studies using electron microscopy suggested that the secretory apparatus itself is polarized toward sites of active growth (PREUSS *et al.* 1992). Therefore, proteins of the endoplasmic reticulum and/or Golgi may interact with the actin cytoskeleton to promote polarized secretion. It is clear from our studies that polarization of actin is necessary but not sufficient by itself to regulate growth polarity since we have been able to identify mutants defective in this process that do not exhibit any obvious defects in the structure of the actin cytoskeleton or in the timing of actin polarization at the G1/S transition. These mutants, therefore, are likely to define complementary functions of the secretory apparatus. Analysis of the mutants suggested that outer chain elongation of *N*-linked oligosaccharides might be part of a signaling pathway specifically involved in polarized secretion. This hypothesis is similar to the proposed role of *N*-glycosylation in the context of protein sorting in epithelial cells (FIEDLER and SIMONS 1995). It is also likely that polarized growth is determined at the final step of the secretion process, exocytosis, by a direct or indirect interaction between the exocytosis complex, the exocyst, and the actin cytoskeleton. The possibility that this interaction is regulated adds another potential level of control of polarized growth.

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