Abstract. The ellipsoidal shape of the yeast *Saccharomyces cerevisiae* is the result of successive isotropic/apical growth switches that are regulated in a cell cycle-dependent manner. It is thought that growth polarity is governed by the remodeling of the actin cytoskeleton that is itself under the control of the cell cycle machinery. The cell cycle and the morphogenesis cycle are tightly coupled and it has been recently suggested that a morphogenesis/polarity checkpoint control monitors bud emergence in order to maintain the coupling of these two events (Lew, D. J., and S. I. Reed. 1995. *J. Cell Biol.* 129:739–749). During a screen based on the inability of cells impaired in the budding process to survive when the morphogenesis checkpoint control is abolished, we identified and characterized *BED1*, a new gene that is required for efficient budding. Cells carrying a disrupted allele of *BED1* no longer have the wild-type ellipsoidal shape characteristic of *S. cerevisiae*, are larger than wild-type cells, are deficient in bud emergence, and depend upon an intact morphogenesis checkpoint control to survive. These cells show defects in polarized growth despite the fact that the actin cytoskeleton appears normal. Our results suggest that Bed1 is a type II membrane protein localized in the endoplasmic reticulum. *BED1* is significantly homologous to *gma12*+, a *S. pombe* gene coding for an α-1,2-galactosyltransferase, suggesting that glycosylation of specific proteins or lipids could be important for signaling in the switch to polarized growth and in bud emergence.

The ellipsoidal shape of the yeast *Saccharomyces cerevisiae* reflects cell cycle-regulated polarized growth. At specific times during the cell cycle, cell growth is either isotropic or polarized toward the bud (for review see Lew and Reed, 1995b). A correlation between local deposition of new cell wall components and actin localization has been established (Adams and Pringle, 1984; Killman and Adams, 1984), leading to the proposal that actin directs secretory vesicles to specific regions of the plasma membrane to allow localized cell surface growth during bud initiation and bud growth. During most of the G1 phase, growth is isotropic and cortical actin patches are delocalized throughout the cell. The attainment of a critical cell size and concomitant execution of START lead to the formation of an actin ring at the pre-bud site and the orientation of actin filaments toward this site. Subsequent to START, growth is almost completely restricted to the emerging bud. During bud growth, cortical actin patches are localized to the bud. Initially, bud growth occurs primarily at the distal tip. At some point, though, there is a switch to isotropic growth first in the bud, then also transiently in the mother cell at mitosis. At cytokinesis, the actin cytoskeleton is reorganized and actin patches are relocalized to the mother-daughter neck where the cell wall is modified for cell separation. The mechanisms by which actin mediates polarized secretion are not well understood, but it has been shown that cortical actin patches are associated with the cell surface through an invagination of the plasma membrane (Mulholland et al., 1994) and it has been suggested that components of the secretory pathway (endoplasmic reticulum [ER] and Golgi) could be transported into the bud to direct localized growth presumably via an actin-dependent mechanism (Preuss et al., 1992).

A variety of proteins have been shown to be required for either bud emergence or for bud site selection (for recent reviews see Bretscher et al., 1994; Chant, 1994; Welch and Drubin, 1994). *CDC42*, encoding a small GTP-binding protein, and several genes encoding its regulators are involved in bud emergence: cells mutated in these genes arrest as large unbudded cells with a disorganized actin cytoskeleton and delocalized chitin. The *BUD* genes, along with *CDC24* and *RSV167* are involved in the selection of the bud site.

In *S. cerevisiae*, the budding cycle is tightly coupled to the central events of the cell cycle. Upon completion of the primary G1 restriction event known as START, when the
mother cell has reached a critical size, bud emergence and
S phase are initiated (Nasmyth, 1993; Reed, 1992). Moreover,
the dramatic changes of actin organization and the
isotropic/apical growth switches observed during the cell
cycle have been shown to be triggered by the different
forms of the Cdc28 kinase that constitute the cell cycle
clock (Lew and Reed, 1993). Furthermore, it has been recen-
tly suggested that growth polarity or bud emergence
are monitored to ensure that mitosis does not occur before
a bud is produced to receive the daughter nucleus (Lew
and Reed, 1995a). The impairment of growth polarity ei-
ther by mutation or external stimuli such as osmotic shock
is detected and results in a G2 delay. This morphogenesis/
polarity checkpoint control is mediated via a partial inhibi-
tion of transcription of the mitotic cyclin genes CLB1 and
CLB2 and also through a more direct inhibition of the mi-
totic form of the Cdc28 kinase via negative regulatory
phosphorylation of Tyr19 of Cdc28 (Lew and Reed, 1995a).
This conserved tyrosine has been shown to be the target of
negative regulatory phosphorylation for a number of dif-
cent cyclin-dependent kinases.

In this paper, we describe the identification and the
characterization of a new gene called BED1 (which stands
for Bud Emergence Delay) 1 that is required for efficient
polarized growth and is important for bud emergence. The
bed1-1 mutation was isolated based on synthetic lethality
with overexpression of mitotic cyclins, a phenotype that
occurs because bed1 mutant cells depend on the morpho-
genesis/polarity checkpoint which overexpression of mi-
totic cyclins overrides. We have shown that the Bed1 pro-
nuclear protein is an integral membrane protein localized in the
endoplasmic reticulum. This protein shares homology with a
previously described S. pombe α-1,2-galactosyltransferase.

Our results suggest that glycosyl modification could play a
role in regulating growth polarity and bud emergence.

Materials and Methods

Yeast Strains, Media, and Growth Conditions

All strains used in this study were derivatives of BY264-15DU: MATa
ade1, his2, leu2-3,112, trp1-1, ura3Dns (Richardson et al., 1989). The rele-
vant genotypes of strains used in this study are shown in Table I. Yeast
cultures were grown at 30°C in YEP (1% yeast extract, 2% bactopeptone,
0.005% adenine, 0.005% uracil) supplemented with 2% glucose (YPEP),
raffinose (YPEP), or galactose (YPEG). Genes under control of the
GAL1 promoter were induced by the addition of 2% galactose to a mi-
dog phase culture (YPEP) for 4 h.

Identification and Molecular Characterization of the
BED1 Gene

A strain carrying a GAL1:CLB2 allele (GY-1) was mutagenized by ultra-
violet radiation (70% death) on YEPD plates (GAL1 promoter re-
pressed) and incubated at 30°C for 2 d. The colonies were then repli-
cated to YEPG (GAL1 promoter induced). Out of 25,000 colonies
screened, 20 were unable to grow on galactose. Based on the level of Clb2
overexpression, we discarded 11 candidates that showed low levels of
Clb2 protein after 4 h galactose induction of the GAL1:CLB2 allele, pre-
sumably because the mutations affected the galactose pathway. The nine
remaining candidates were then crossed to the wild-type 15D strain and
the resulting tetrad were analyzed to show that, for eight of them, the le-
thality was associated with overexpression of Clb2. The mutant strains
were backcrossed to a MATa-GAL1:CLB2 strain (GY-101) and the dip-
loid strains were then induced to sporulate and meiotic asci dissected to
verify that the lethality on YEPG was due to a single mutation and that
the mutations were not localized to the GAL1:CLB2 locus. A comple-
mentation analysis with the 8 remaining candidates showed that they be-
long to 6 different complementation groups, 2 of them with 2 alleles. We
analyzed in greater detail one of them, that contains one allele, bed1-1.

The BED1 gene was cloned by complementation of the lethality of the
strain GY-159 (GAL1:CLB2-bed1-1) on YEPG plates with a YCP50
based genomic yeast DNA library (Rose et al., 1987). The screening of
15,000 transformants (the equivalent of 10 genomes) yielded the plasmid
pR159.1 5 times and the plasmid pR159.5 a single time, containing inserts

Table I. Yeast Strains

| Strain  | Genotype                                      | Source*       |
|---------|----------------------------------------------|---------------|
| 15Daub  | MATa-ade1-his2-leu2-3,112-trpl-1*-ura3Dns-bar1Δ | S. I. R.      |
| DLY-005 | MATaα                                         | D. J. L.      |
| GY-1    | MATa-GAL1-CLB2(LEU2)                         | S. I. R.      |
| GY-101  | MATa-GAL1-CLB2(LEU2)                         | This study    |
| GY-159  | MATa-GAL1-CLB2(LEU2)-bed1-1                  | This study    |
| GY-381  | MATa-bed1::URA3                             | This study    |
| GY-382  | MATa-bed1::URA3-GAL1-CLB2(LEU2)              | This study    |
| GY-409C | MATa-bed1::URA3-GAL1-CLB2(LEU2)              | This study    |
| GY-449  | MATa-bed1::URA3-cdc28-LEU2-cdc28G94A18(Trp1) | This study    |
| GY-488  | MATa-bed1-1                                  | This study    |
| GY-489  | MATa-bed1-1::BED1(LEU2)                      | This study    |
| GY-647  | MATa-bed1-1::URA3-bed1::ura3:LEU2           | This study    |
| GY-650  | MATa-bed1-1::BED1(LEU2)                      | This study    |
| GY-651  | MATa-bed1-1::BED1(LEU2)-bed1-1               | This study    |
| GY-711  | MATa-bed1-1::BED1(LEU2)                      | This study    |
| GY-773  | MATa-bed1-1::URA3-GAL1-BED1(HA)3X(LEU2)      | This study    |
| GY-716  | MATa-bed1-1::URA3-GAL1-BED1(LEU2)            | This study    |
| GY-718  | MATa-bed1-1::URA3-GAL1-BED1(HA)3X(URA3)      | This study    |
| GY-721  | MATa-bed1-1::URA3-GAL1-BED1(LEU2)            | This study    |
| GY-723  | MATa-bed1-1::URA3-GAL1-BED1(HA)3X(LEU2)      | This study    |
| GY-748  | MATa-gal10Δ                                  | This study    |
| GY-755  | MATa-BED1::BED1(LEU2)                        | This study    |

* S. I. R., Steven I. Reed; D. J. L., Daniel J. Lew.
of 12 kb and 9 kb, respectively (see Fig. 7A). These two DNA fragments had an overlap a region of 2.7 kb that was able to rescue the lethality of the strain GY-159 on YEPG medium. This region was sequenced on both strands using an automated sequencing system (Applied Biosystems, Foster City, CA).

A diploid strain BED1/bed1-1::BED1(LEU2) (GY-650; see below) was constructed, induced to sporulate, and 48 tetrad were analyzed and 47 parental ditype (PD): 0 nonparental ditype (NPD); 1 tetratype (TT) were observed demonstrating a tight linkage between bed1-1 and LEU2 loci (~1 cm). We also created a diploid strain BED1::BED1(LEU2)/bed1-1 (GY-651; see below), dissected 23 tetads and 23 PD: 0 NPD: 0 TT were recovered, indicating again that the gene cloned by complementation was likely to correspond to the BED1 locus defined mutationally.

Plasmids

The 2.7-kb DNA fragment defined by the region of overlap between plasmids pR159.1 and pR159.5 was subcloned into pBlueScript (Stratagene, La Jolla, CA) and the BED1 gene was disrupted by replacing an 0.8-kb XbaI fragment within the coding region with a HindIII DNA fragment containing the URA3 gene to give the plasmid pBS159:URA3 (see Fig. 1B). The BED1 ORF was amplified by PCR using primers containing BamHI sites at each end. The BamHI site before the STOP codon (primers: 5'-CCC-

Plasmids

GCGGCCGCCTGGGAAGAAAAATCTCGTGT-3'; the BamHI and NotI sites are indicated in bold characters and the ATG and STOP codons were preserved. Other plasmids were constructed by standard genetic procedures (Sambrook et al., 1989).

Strain Construction

Strains were constructed according to standard genetic procedures (Sherman et al., 1982) except that transformations of yeast cells were performed as described by Ebelle (1992). The strain GY-381, disrupted for the LEU2 gene was disrupted by transplacement of a mutated version of LEU2 into the LEU2 site of the plasmid pBM58(URA3) (a generous gift from Mark Johnston) linearized with PvuII; gal- colonies were then recovered on 5-fluor-orotic acid (FOA) plates to select for transplacement.

Protein Analysis

Protein extracts and cell fractionation were performed as described previously (Graham et al., 1994). Cells from mid-log phase cultures were spheroplasted with Zymolase and lysed to obtain total protein extracts with intact membrane structures. These extracts were then centrifuged for 15 min at 13,000 g to generate the P13 fraction and the supernatant was centrifuged for 10 min at 105,000 g to generate the S100 fraction. Levels of the HA-tagged fusion proteins were detected by Western blotting using the 12CA5 monoclonal antibody (Boehringer Mannheim Corp., Indianapolis, IN) at a concentration of 5 ng/ml. The secondary anti-body was an FITC-conjugated goat serum directed against whole mouse IgG. Protein extracts and cell fractionation were performed as described previously (Graham et al., 1994). Cells from mid-log phase cultures were spheroplasted with Zymolase and lysed to obtain total protein extracts with intact membrane structures. These extracts were then centrifuged for 15 min at 13,000 g to generate the P13 fraction and the supernatant was centrifuged for 10 min at 105,000 g to generate the S100 fraction. Levels of the HA-tagged fusion proteins were detected by Western blotting using the 12CA5 monoclonal antibody (Boehringer Mannheim Corp., Indianapolis, IN) at a concentration of 5 ng/ml. The secondary anti-body was an FITC-conjugated goat serum directed against whole mouse IgG.
totic form of Cdc28 kinase. This situation would be analogous to the lethality observed when a nondegradable form of Clb1 (Clb1Δ152) is overexpressed in wild-type cells (Ghiara et al., 1991). A second expected class of mutation conferring a defect in bud emergence or growth polarity can also be obtained with this screening method. Delays or blocks in generation of growth polarity or in bud emergence are detected by a morphogenesis checkpoint leading to a temporary G2 arrest (Lew and Reed, 1995a). This control can be overridden either by mutation of tyrosine 19 of the Cdc28 kinase or the overexpression of mitotic cyclins Clb1 or Clb2 (Lew and Reed, 1995a). Therefore mutations that delay budding or the generation of growth polarity could confer a lethal phenotype when \textit{CLB2} is overexpressed due to abrogation of the checkpoint. In this situation, cells dependent on the checkpoint would die with more than one nucleus as a result of mitosis occurring.
before budding. Eight mutations belonging to six complementation groups were isolated from the screening of 25,000 mutagenized colonies (see Materials and Methods for more detailed description of the screening procedure). The first mutation that we characterized was a mutated allele of the previously described VRP1 gene (Donnelly et al., 1993). This gene encodes verprolin, a proline-rich protein required for proper actin organization. The fact that we recovered VRP1, a gene presumably involved in growth polarity and bud emergence, indicated that the screening method was effective for identification of genes involved in growth polarity and bud emergence. We then studied in greater detail the bed1-1 mutation; the gene defined by this mutation and the encoded protein are the subject of this paper.

**A bed1::URA3 Strain Is Defective in Bud Emergence and the Morphogenesis Checkpoint Is Necessary for Its Viability**

Although a GAL1:CLB2-bed1-1 strain was able to grow on dextrose medium (GAL promoter repressed) and not on galactose medium (GAL promoter induced), a bed1-1 strain was viable on both media. FACS analysis of nuclear DNA content of a strain overexpressing Clb2 in a bed1-1 background showed that a large fraction of the cells were arrested with a 4N DNA content (Fig. 1) while Clb2 overexpression in a wild-type background induced only a delay in mitosis (Stueland et al., 1993). Microscopic observation revealed that a large proportion of the cells had more than two nuclei (see below for detailed analysis of the bed1 phenotype). The *BED1* gene was cloned, sequenced, and a null mutation in the *BED1* gene was created by the one-step disruption method. The cloned gene was shown to be genetically linked to the *BED1* locus (see Materials and Methods and below). We observed that the mutation and the targeted disruption conferred similar phenotypes and therefore used the bed1::URA3 strain to investigate the bed1 phenotype in detail. This strain was viable but showed a 50% reduction in growth rate in rich (YEPD) liquid medium compared to the isogenic wild-type strain. The cells were larger than wild-type and almost completely round, having lost the ellipsoidal morphology characteristic of *S. cerevisiae* (Fig. 2 B). Using a Coulter Channelizer, bed1::URA3 cells were shown to be 50% larger than wild-type cells in rich medium (the mean cell volume for bed1::URA3 cells was 64 fl vs 42 fl for wild-type cells). bed1::URA3 cells also had defects in cell separation, in that cultures contained clumps of aggregated cells which could not be completely disrupted by sonicication (see Fig. 2 B). However, treatment with the cell wall-digesting enzyme Zymolyase gave single cells (not shown), indicating that the defect was in cell separation rather than cytokinesis. FACS analysis of bed1::URA3 cells showed an increase in the proportion of S/G2/M cells in an asynchronous culture in rich liquid medium. A small fraction of the cells scored as greater than 2N in DNA content, presumably because of the cell separation defect (Fig. 2 A). In wild-type cells, bud emergence is concomitant with the beginning of S phase as is illustrated by the correlation between the budding index and the percentage of cells that have entered or completed S phase. This was not the case in bed1::URA3 mutants: in YEPR medium, the percentage of budded cells was lower than the percentage of cells that had entered or completed S phase despite the fact that the budding index was most likely overestimated due to the excessive aggregation associated with the strain (Fig. 2 A). These observations were suggestive of a defect in bud emergence conferred by the bed1::URA3 mutation.

To further characterize this phenotype, small G1 wild-type and bed1::URA3 cells were isolated by centrifugal elutriation, inoculated into fresh YEPD medium and execution of START, initiation of S phase, bud emergence, and nuclear division were followed (Fig. 3). As previously described (Lew et al., 1992), in the 15D wild-type background, completion of START was followed by S phase and bud emergence within 15–20 min. Nuclear division then occurred 45–50 min later. In bed1 disruptant cells,
completion of START occurred sooner than in wild-type cells, presumably because the elutriated population exhibited a larger size than the wild-type controls (not shown). S phase began normally 15–20 min after START but bud emergence was delayed by more than an hour. Nuclear division took place very rapidly after bud emergence, within 10–15 min, suggesting that the morphogenesis checkpoint delays mitosis only until a bud forms to receive the daughter nucleus. Only a small fraction of bed1::URA3 cells contained more than one nucleus whereas most of the cells overexpressing Clb2 in this background became multinucleated (Fig. 4). This is consistent with the fact that the overexpression of Clb2 or Clb1 is lethal in bed1-1 or bed1::URA3 cells (Fig. 1 A and Fig. 2 B; not shown). In a synchronized culture, these cells went through S phase and mitosis before bud emergence and died with more than two nuclei (not shown). The disruption of the BEDI gene in the context of the cdc28\(^{F19A18}\) mutation (where CDC28 is mutated so as to be no longer subject to negative regulatory phosphorylation) was not lethal but a large fraction of the cells were multinucleated (Fig. 4). It was difficult to quantify the percentage of multinucleated cells in these different strains because of the cell aggregation phenotype, but it was clear that more multinucleated cells were detected when Clb2 was overexpressed or Cdc28 was not phosphorylatable. It was also apparent that, in bed1::URA3 cells, nuclear division occurred very rapidly after bud emergence, in that daughter nuclei were observed even in very small buds while nuclear division took place in wild-type cells when buds were much larger (Fig. 4). We

![Image of DAPI stained cells](image-url)

**Figure 4.** The viability of bed1::URA3 cells depends on the integrity of the morphogenesis checkpoint control machinery. Nuclei were visualized by staining cells with DAPI: (A) wild-type (15Daub in YEPD); (B) bed1::URA3 (GY-381 in YEPD); (C) bed1::URA3-GAL1:CLB2 (GY-382 after a 4 h induction with 2% galactose in YEPR); (D) bed1::URA3-cdc28::LEU2-CDC28\(^{F19A18}\)(TRP1) (GY-449 in YEPD). Note that the fields are not representative of the percentage of multinucleated cells in the different strains. Magnification is the same for all the strains. Bar, 10 μm.
concluded from these experiments that the BED1 gene was required for timely bud emergence, that the morphogenesis checkpoint is functional and that the viability of bed1::URA3 cells depends on the integrity of this checkpoint control machinery.

**bed1::URA3 Cells Have Defects in Polarized Growth Although Actin Is Properly Polarized**

The fact that bed1 mutant cells are delayed in bud emergence, are large, and have an unusually round morphology, suggested that they might be defective in polarized growth during the cell cycle. This type of phenotype is often associated with an inability to properly organize the actin cytoskeleton (see Discussion). We investigated this possibility by looking at actin localization by rhodamine-phalloidin staining of bed1 mutant cells. As shown in Fig. 5A, actin staining revealed a pattern similar to that observed in wild-type cells: actin rings were observed at the pre-bud site and after bud emergence, actin patches were found exclusively in the buds and actin cables were oriented toward the tips of the buds. Finally actin patches

---

**Figure 5.** Actin and chitin localization in bed1::URA3 cells. Wild-type (15Daub) and bed1::URA3 (GY-381) cells grown in YEPD were fixed with formaldehyde and stained with rhodamine-phalloidin to visualize actin (A) or with calcofluor to detect chitin (B). Magnification and exposure time are the same for both strains. Bar, 10 μm.
were relocalized to the necks of the buds during cytokinesis. We also observed, as with wild-type cells, that actin patches were concentrated to the tip of the growth projection (shmoo) when bed1 mutants were treated with the mating pheromone α factor (not shown).

We then determined the timing of polarization of the actin cytoskeleton during the cell cycle in a synchronized culture (Fig. 3). bed1 mutant cells began to undergo actin polarization within 5 min after completing START, as in wild-type cells. We concluded, therefore, that actin was properly polarized at the appropriate time during the cell cycle, indicating that the bed1 phenotype does not result from an inability to reorganize the actin cytoskeleton at the G1/S phase boundary.

On the other hand, staining of bed1 mutants with calcofluor, a stain for chitin, which is normally found concentrated in the neck region of a budded cell and in “scars” on cells where previous buds were located, revealed that chitin was now completely delocalized and deposited at elevated levels (Fig. 5 B). One interpretation of this phenotype is that bed1 mutants are defective in polarized secretion required for proper localization of chitin synthase despite the fact that there is no obvious defect in polarization of the actin cytoskeleton.

Growth polarity can be directly visualized by pulse-labeling cells with FITC-ConA, which binds mannose residues from the cell wall and chasing in the absence of FITC-ConA (for a more detailed description of this method, see Lew and Reed, 1993). Exponentially growing wild-type and bed1::URA3 cells were pulse labeled and fixed after a chase of one generation equivalent in fresh medium. Daughter cells originating from labeled buds (cells with an unlabeled birth scar) were scored for staining patterns characteristic of isotropic growth (uniform staining; see, for example, cell 1 in Fig. 6 A) or apical growth (staining that fades out toward one end of the cell; see, for example, cell 2 in Fig. 6 A). As previously described (Lew and Reed, 1993), ~40% of the daughter cells from a wild-type population in YEPD medium exhibited polarized growth (169 out of 449 daughter cells). Among the cells exhibiting a pattern indicative of polarized growth, 15% showed a partial gradient of the staining where the pole of the cell was still detected (see cell 3 in Fig. 6 A; 27 out of 169). On the other hand, most of the bed1::URA3 daughter cells (~60%; 275 out of 452 daughter cells) exhibit a uniform staining indicative of completely isotropic growth (cell 4) and no daughter cells with completely unlabeled poles indicative of apical growth were observed. However, ~15% of these cells (66 out of
Figure 7. Identification and molecular cloning of the **BED1** gene. (A) Physical map of the DNA fragment able to rescue the lethality on galactose of the **GY-159** strain. The disruption of the **BED1** gene with the **URA3** marker is also schematized. (B) Sequence of the **BED1** gene with the **URA3** marker is also schematized. (C) Sequence of the **BED1** gene with the **URA3** marker is also schematized.

452) exhibited a partial fade-out staining with a decreasing gradient of the staining to one end of the cell but with the pole still labeled (see for example, cell 5 in Fig. 6 A) and ~25% (111 out of 452) showed a faint uniform staining with a zone of strong staining around the birth scar (see, for example, cell 6 in Fig. 6 A). We used confocal microscopy to analyze the fade-out staining in greater detail (Fig. 6 B). While no staining could be detected at the opposite pole of the unlabeled birth scar in wild-type cells, the end of **bed1::URA3** daughter cells was always stained, suggesting that...
the apical growth, when it occurs, is defective or incomplete. Examination of cells with a stronger signal around
the birth scar (cell 6 in Fig. 6 A) showed that the pattern is
consistent with staining of a region very close to the scar it-
self but without any gradient toward the pole of the cell.
Our interpretation is that growth cannot occur in this re-
region for mechanical reasons and therefore the signal can-
ot be diluted during bud growth and appears as a narrow
ring of heavily stained cell wall. The fact that we could still
detect in some cells a partial gradient toward one pole of
the cell (~15% of daughter cells) suggests that growth
may be partially polarized at some point of the budding
phase. Taken together, these results suggest that bedh:
URA3 cells exhibit some growth polarity but that apical
growth is not as efficient as in wild-type cells.

Molecular Characterization of the BED1 Gene:
BED1 Shows Similarity to an S. pombe Gene Encoding
an α-1,2-Galactosyltransferase

The gene encoding Bed1 was cloned by complementation
of the GAL1:CLB2-bedl-1 mutant strain (GY-159) using
a centromeric yeast genomic DNA library and two plas-
mids containing a 2.7-kb overlapping region were recov-
ered (Fig. 7 A). We detected one large open reading frame
(ORF) in this region with a capacity for encoding a protein
of 393 amino acids (Fig. 7 B). Examination of the se-
quence of the BED1 gene showed a stretch of hydropho-
bic amino acids in the NH2-terminal portion of the ORF
(residues 47 to 67; Fig. 7 B). The inferred structure of
Bed1 was reminiscent of the organization of type II mem-
brane proteins: a short NH2-terminal cytosolic domain, a
unique hydrophobic transmembrane domain (15–20 ami-
noacids) and a large luminal COOH-terminal domain (for
review see High and Dobberstein, 1992).

BED1 had similarity to the recently cloned gma12+ gene
of S. pombe (Chappell et al., 1994). The two proteins are
similar in their predicted luminal domains: 4 regions that
show ~30% identity and up to 70% similarity (Fig. 8). The
structures of these two proteins are different in that, al-
though they both have type II membrane protein structure,
the cytoplasmic domain of Bed1 is larger (45 amino
acids) than the corresponding domain of gma12p (only
two amino acids amino terminal to the transmembrane do-
main). The gma12+ gene encodes an α-1,2-galactosyltras-
ferase involved in the synthesis of the S. pombe cell wall
which, in contrast to that of S. cerevisiae, contains glyco-
proteins with galactose residues. More recently, two puta-
tive ORF homologous to gma12p were detected on chro-
mosome I of S. pombe. Therefore, gma12+ belongs to a
highly conserved family of proteins that are conserved
along their entire lengths in the fission yeasts S. pombe
and S. octosporus (Fig. 8; Chappell, T., personal communi-
cation).

We also noticed that Bed1 is even more homologous to a
previously unidentified S. pombe partial ORF present
adjacent to the vacuolar H+-ATPase, subunit B gene (Fig.
8). Analysis of the sequences available shows that the ho-
mology between Bed1 and this partial ORF is significantly
greater than that between Bed1 and gma12p (see Fig. 8).
The structure of this ORF is also more similar to the struc-
ture of Bed1 in that the putative cytoplasmic domain con-
tains ~40 amino acids. However, the role of the protein rep-
resented in part by this ORF is not known. The possible
implications of these homologies will be discussed below.

Bed1 Is a Type II Membrane Protein Localized in the
Endoplasmic Reticulum

Since the analysis of bed1::URA3 cells suggested a role in
polarized secretion and Bed1 had a predicted structure or-
ganization consistent with a membrane protein, we deter-
mined the intracellular localization of Bed1. We intro-
duced a COOH-terminal triple influenza hemagglutinin
([HA]3X)-tagged version of Bed1 into bedh:URA3 cells.

Figure 8. Homologies between BED1 and gma12+, an S. pombe gene encoding an α-1,2-galactosyltransferase. The search for sequences
homologous to Bed1 was performed at the National Center for Biotechnology Information (NCBI) through the GENINFO (R) BLAST
Network Service (Blaster) (Altschul et al., 1990). Four S. pombe ORFs were identified: gma12p (SPA12GATR; accession number
z09174), two ORFs on chromosome I (cds11 and cds13; accession number z49811) and a previously unidentified partial ORF (spORF)
located 5' from the vacuolar H+-ATPase, subunit B gene (SPVATPB). The alignment of the 5 ORFs was established with the following
rules: G=A=P=S; S=A=T; R=H=K; D=E; Q=N; M=L=V=F; F=W=Y.
coupling between these two events in place immediately after bud emergence, indicating a tight occurring only 1 h after initiation of S phase in other related gene. Bud emergence was strongly delayed, the transferase and that the switch to polarized secretion might require galactosyl modification of particular proteins or lipids during transit through the endoplasmic reticulum. Some residual growth polarity was observed, explaining why bedl::URA3 cells are impaired in directing secretory vesicles to the bud site but not in secretion per se, and that Bed1 is part of a pathway that is downstream or parallel to the actin pathway; both pathways being necessary for proper delivery of secretory vesicles to the bud site during bud emergence and to the bud neck during cytokinesis and cell separation.

A role in polarized secretion is also consistent with the intracellular localization of Bed1. Crude fractionation of whole cell lysates showed that Bed1 was present in a low speed fraction (P13) enriched for endoplasmic reticulum, nuclear envelope, vacuoles, and plasma membrane. Kar2, a luminal protein of the ER (Rose et al., 1989), was also mainly present in this fraction, as expected. We also showed that, unlike Kar2, Bed1 was tightly associated with membranes: Bed1 remained membrane associated after treatment of the membrane fraction with carbonate pH11.0 but not after treatment with detergents (not shown). Moreover, only the NH$_2$-terminal portion of Bed1 was sensitive to proteolysis by proteinase K when the protein was associated with intact membranes, suggesting that the first 45 amino acids are likely to be cytosolic in intact cells (not shown). Based on these criteria, we concluded that Bed1 is a type II integral membrane protein.

We were unable to detect Bed1[HA]3X by immunofluorescence when the fusion protein was expressed under the control of its native promoter (not shown). Therefore, we constructed a strain containing the tagged protein under control of the constitutive GAP promoter (GY-718), yielding a 20-fold increase in the amount of Bed1 protein, as observed by Western blot (Fig. 9 A). Since it has been shown that, in some cases, overexpression of proteins of the secretory pathway can lead to a mislocalization due to saturation effects, we verified that Bed1 had the same fractionation profile when overexpressed as when it was expressed under control of its own promoter (Fig. 9 A). We observed an immunofluorescence staining pattern consistent with an ER localization of the protein: the signal was perinuclear with some extensions into the cytoplasm. This pattern was similar to the immunolocalization of Kar2, a luminal protein of the endoplasmic reticulum (Rose et al., 1989). We also observed that Bed1 colocalized with Kar2 in individual cells (not shown). These data taken together suggest that Bed1 is a type II integral membrane protein of the endoplasmic reticulum and support the hypothesis, based on homologies with gna12+, that Bed1 is a galactosyltransferase and that the switch to polarized secretion might require galactosyl modification of particular proteins or lipids during transit through the endoplasmic reticulum. However, bedl cells became multinucleated when the mitotic form of Cdc28 was hyperactivated by either the overexpression of the mitotic cyclins Clb1 or Clb2 or by mutation of Tyr19, the regulatory phosphorylation site of Cdc28. Lew and Reed (1995a) have shown that, while the G2 delay induced by defects in growth polarity or budding can be completely abolished by Clb1 or Clb2 overexpression, the cdc28G2 mutation only reduces the delay but cannot eliminate it completely. This accounts for the observation that a bedl::URA3-cdc28G2 strain is viable while overexpression of Clb2 in a bedl::URA3 background is lethal. Taken together, these observations suggest that the viability of bedl cells depends on the morphogenesis checkpoint machinery which is able to delay mitosis in the absence of budding in order to maintain the coordination between the nuclear division cycle and the budding cycle. In fact, the dependence of the bedl mutant on the morphogenesis checkpoint for survival is the most convincing demonstration of the importance of this regulatory system in the yeast life cycle. Finally, cells disrupted for BED1 exhibited morphological aberrations, losing the ellipsoidal shape characteristic of S. cerevisiae and being larger than wild-type cells. In addition, bedl cells had defects in cell separation as indicated by a tendency to form aggregates in liquid medium.

The delayed bud emergence, the morphological and morphogenetic phenotypes observed in bedl::URA3 cells (increased size, round cell shape, and delay in bud emergence) could be a result of defects in secretion or the generation of growth polarity by analogy with other morphogenesis mutants (for reviews see Bretscher et al., 1994; Welch and Drubin, 1994). Disorganization of the actin cytoskeleton and delocalized deposition of chitin are usually phenotypically coupled, presumably because proper actin function is required for polarized secretion and therefore for budding. In bedl::URA3 cells, actin polarization after START and actin reorganization at cytokinesis occurred on schedule. Surprisingly, however, chitin deposition was greatly increased and completely delocalized, indicating that bedl cells have defects in polarized growth. Moreover, a more direct evaluation of growth polarity by in vivo pulse labeling with FITC-ConA showed that bedl::URA3 cells are defective in the most polarized form of growth, growth directed to the bud tip or apical growth. These observations, we suggest that bedl cells are impaired in directing secretory vesicles to the bud site but not in secretion per se, and that Bed1 is part of a pathway that is downstream or parallel to the actin pathway; both pathways being necessary for proper delivery of secretory vesicles to the bud site during bud emergence and to the bud neck during cytokinesis and cell separation.
Figure 9. Bed1 is a membrane protein localized in the endoplasmic reticulum. (A) Bed1 is present in an ER-enriched fraction. Total protein extracts (TOTAL) containing intact membrane structures were subjected to a crude fractionation procedure: the P13 fraction is enriched for membranes from the endoplasmic reticulum, the vacuoles, the plasma membrane and the nuclear envelope; the P100 fraction is enriched for Golgi membranes; the S100 fraction contains soluble proteins. The Bed1[HA]3X protein was detected by Western blot with the 12CA5 mouse monoclonal antibody and Kar2 with a rabbit polyclonal antibody. Note that twice as much volume of GY-723 (GAP:BED1[HA]3X) was loaded compared to GY-718 (GAP:BED1[HA]3X). It was estimated by densitometric scanning of different exposures of the Western blots that 40% of Kar2 was in the P13 fraction, 20% in the P100, and 20% in the S100 fraction. The same kind of measurement gave for Bed1 90%, 10%, and less than 1% in fractions P13, P100, and S100, respectively. (B) Immunolocalization of Bed1. GY-718 (containing GAP:BED1[HA]3X) and GY-716 (containing GAP:BED1 as a negative control) cells grown in YEPD were stained with DAPI to visualize the nuclei and at the same time with the 12CA5 antibodies to detect the Bed1 fusion protein. Magnification and exposure time are the same for both strains. Bar, 5 μm.
domain and COOH-terminal luminal domain. The predicted structure of the protein was in agreement with this conclusion since Bed1 contained a putative hydrophobic transmembrane domain in the NH2-terminal portion of the protein (amino acids 47–67). We were not able to detect an epitope-tagged version of the protein expressed from its own promoter by immunofluorescence because of low levels of expression but a staining pattern suggesting an ER localization for Bed1 was obtained when the tagged protein was overexpressed. We showed however that the behavior of Bed1 in the fractionation procedure we used was not affected by overexpression from the constitutive GAP promoter. Taken together, these two different approaches suggested that Bed1 is an integral type II membrane protein of the ER.

We discovered recently that the \textit{BED1} gene was independently cloned as \textit{SLC2} (Karpova et al., 1995). \textit{slc2} mutants were identified during a screen designed to identify mutations synthetically lethal with a disruption of \textit{CAP2}, a gene involved in actin cytoskeleton organization (Karpova et al., 1993). The phenotype associated with the \textit{slc2-107} is different from that described here: the actin cytoskeleton is disorganized in a strain carrying the \textit{slc2-107} allele and this strain is thermostable while \textit{bed1::URA3} cells have a normal pattern of actin polarization and are not temperature sensitive (at least up to 37°C, not shown). This could be explained either by strain background differences or by the fact that the \textit{slc2-107} mutation is semidominant, suggesting that this mutation might be associated with a gain-of-function. More recently, the sequence of the \textit{BED1} gene appeared twice in the Genbank database: it was detected during the sequencing of chromosome IV and \textit{BED1} is identical to \textit{MNN10} (accession number I42540). \textit{mnn} mutants were isolated as mutants that have aberrant carbohydrate structures in the cell wall; most of the gene products are thought to be involved in mannosylation of proteins but some could be involved in more general functions of the secretory pathway that might affect mannosylation indirectly (for review see Hercovics and Orlean, 1993). This latter hypothesis is consistent with our results. \textit{BED1} was found to be similar to 4 ORFs in \textit{S. pombe}, \textit{gma12}+ and 2 of its homologues and a previously unidentified ORF we called spORF. The homology between Bed1 and \textit{gma12p/cds11/cds13} was particularly significant over 4 regions in the luminal portion of these proteins (~30% identity and 65% similarity). The \textit{gma12p} protein has been shown to be an α-1,2-galactosyltransferase (Chappell et al., 1994). Several galactosyltransferase activities have been detected in \textit{S. pombe} (Chappell et al., 1994; Ballou and Ballou, 1995) and the \textit{gma12}+ gene belongs to a large family of related genes in \textit{S. pombe} (Chappell, T., personal communication). Although the structures of the two proteins are clearly similar, Bed1 has a larger cytoplasmic domain. On the other hand, this domain is comparable in size with the corresponding domain of spORF and, furthermore, comparison of the available sequences of spORF and Bed1 (Fig. 7) showed that the degree of similarity between Bed1 and spORF was higher than between Bed1 and \textit{gma12p} (~40–50% identity and 70% similarity). We conclude from these observations that spORF is more likely than \textit{gma12p} to be the homologue of \textit{BED1} in \textit{S. pombe}. Indeed, \textit{gma12}+ could not complement the morphological defects observed in \textit{bed1} cells, although it was shown to be enzymatically active in vitro in extracts from \textit{S. cerevisiae} cells overexpressing \textit{gma12p} under the control of the \textit{GAL1} promoter (not shown). Moreover, localization of \textit{gma12p} in the Golgi apparatus (Chappell et al., 1994) is distinct from that of Bed1. Finally, \textit{gma12p} is involved in bulk modification of proteins of the cell wall, a phenomenon particular to \textit{S. pombe}, that has not been detected in \textit{S. cerevisiae}; we propose that the role of Bed1 is more specific (see below).

It was surprising to find homology between Bed1 and a galactosyltransferase since, to our knowledge, no galactosyl modifications have been described for glycoproteins or glycolipids in \textit{S. cerevisiae}. We were unable to detect any galactosyltransferase activity in extracts from wild-type cells or from cells overexpressing Bed1 under conditions where ectopically overexpressed \textit{gma12p} showed significant activity (not shown). This assay, based on conditions described for \textit{gma12p} (Chappell and Warren, 1992), is somewhat restrictive and does not rule out the possibility that Bed1 could be a galactosyltransferase that cannot use α-methylmannoside or α-methylgalactoside as an acceptor. Furthermore, the idea that Bed1 is a galactosyltransferase is in conflict with the fact that no phenotype has been described in association with disruption of the \textit{GAL10} gene encoding UDP-glucose 4-epimerase, the enzyme responsible for interconversion of UDP-glucose and UDP-galactose. We verified that the disruption of this gene in our genetic background did not confer a morphological phenotype similar to that of \textit{bed1::URA3} cells when grown on glucose medium (not shown). An alternative possibility is that another epimerase is present in \textit{S. cerevisiae} and is responsible for the production of UDP-galactose from UDP-glucose for the purpose of galactosyl modification of specific proteins or lipids. Such modification targeted to specific protein or lipid species could have escaped detection in analysis of bulk glycoproteins or glycolipids. This raises the intriguing possibility that specific galactosyl modification may be involved in signaling the isotropic to polarized switch in secretion. We are currently investigating using a PCR approach the hypothesis that \textit{S. cerevisiae} contains other glucose-4-epimerase(s). Alternatively, Bed1 may catalyze a different glycosyl modification although, based on precedent, this is unlikely: high levels of structural homology have been detected only between enzymes that catalyze analogous glycosylation reactions (Klee and Berger, 1993).

A simple model to explain the role of Bed1 in polarized growth and therefore in bud emergence is that Bed1 is involved in modification of an effector protein that controls the targeting of the secretory vesicles via the actin cytoskeleton in the context of the switch from isotropic to polarized growth at the G1/S phase boundary. In an alternative model, Bed1 might catalyze a modification that leads to local reorganization of the membrane and/or the cell wall at the bud site, allowing vesicles to fuse more efficiently with the plasma membrane. Elucidation of the function of Bed1 will require the identification and characterization of its target(s).

It is interesting that \textit{bed1} mutant cells show defects in polarized secretion and that cell division occurs when buds are unusually small (Fig. 4). One interpretation of this ob-
In the yeast *Saccharomyces cerevisiae*, the cell cycle is a critical process that governs the division of the cell into two daughter cells. The cell cycle is tightly controlled to ensure proper chromosome segregation and cell growth. However, in the yeast *Schizosaccharomyces pombe*, which is a fission yeast, the cell cycle includes a specific phase called the conidiation phase that precedes cell division. This phase is essential for the development of the sporangium, which protects the spores until suitable environmental conditions allow for germination and cell division.

In *S. cerevisiae*, the actin cytoskeleton plays a central role in cell cycle progression and morphogenesis. Actin filaments are involved in various processes such as cell movement, cytokinesis, and vesicle trafficking. The actin cytoskeleton is dynamic, and its organization is regulated by the activity of actin-binding proteins, such as profilins and cofilins, which control actin filament assembly and disassembly.

In contrast, the actin cytoskeleton in *S. pombe* is characterized by a more dynamic and less organized pattern, and the role of actin in cell cycle progression is less well understood. The actin cytoskeleton in *S. pombe* is involved in cell growth, cell division, and cell shape changes, but the mechanisms that control its dynamics and function are less clear than in *S. cerevisiae*.

The differences in actin organization between the two yeasts highlight the unique challenges and adaptations that each yeast species has evolved to ensure efficient cell cycle progression and morphogenesis. Understanding these differences can provide insights into the fundamental principles of cell cycle regulation and actin dynamics, which are essential for the development of new therapeutic strategies for diseases related to cell cycle disorders.