

Filamentous Growth of *Saccharomyces cerevisiae* Is Regulated by Manganese

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Asleson, C. M., Asleson, J. C., Malandra, E., Johnston, S., and Berman, J. 2000. Filamentous growth of *Saccharomyces cerevisiae* is regulated by manganese. *Fungal Genetics and Biology* 30, 155–162. The *Candida albicans* *INT1* gene is a virulence factor that contributes to both adhesion and filamentous growth of the fungus. Expression of *INT1* in the budding yeast *Saccharomyces cerevisiae* directs both adhesion and filamentous growth. Because Int1p contains two predicted divalent cation-binding motifs, we asked whether divalent cations are important for the role of Int1p in filament formation. In this study, we found that *INT1*-induced filamentous growth (I-IFG) is sensitive to the divalent cation chelator EDTA and that this EDTA sensitivity can be ameliorated by the addition of Mn²⁺, but not Mg²⁺ or Ca²⁺ ions. The addition of MnCl₂ restored both the proportion of cells forming filaments and the length of filaments formed. Expression of *INT1* in *S. cerevisiae* mutants that reduce the intracellular concentration of Mn²⁺ did not affect I-IFG. Interestingly, the Mn²⁺ dependence of I-IFG is not dependent upon the presence of the putative divalent cation-binding domains found in *INT1*. Rather, we found that polarized growth induced by mutations in *CDC12* and *CLA4* or by expression of excess *SWE1* was also

sensitive to EDTA treatment and was restored by the addition of MnCl₂ but not by the addition of CaCl₂. Thus, our results suggest that in *S. cerevisiae* polarized growth is dependent upon the presence of Mn²⁺ ions. © 2000 Academic Press

Index Descriptors: polarized growth; morphogenesis; filamentation.

Candida albicans, the most prevalent human fungal pathogen (Odds, 1988) exhibits multiple morphologies, including a round yeast form and several filamentous forms (pseudohyphae, germ tubes, and hyphae). The ability to switch between these morphologies is positively correlated with virulence (Corner and Magee, 1997; Lo *et al.*, 1997; Mitchell, 1998).

The *C. albicans* *INT1* gene, cloned by Hostetter and coworkers (Gale *et al.*, 1996), encodes a protein, Int1p, that has very limited homology to vertebrate integrins, including two putative EF-hand cation binding domains and a single putative metal ion-dependent adhesion site (MIDAS) in the N-terminal end of the protein (Gale *et al.*, 1996). In *C. albicans*, disruption of both *INT1* alleles reduces filamentous growth on several solid media, reduces the adhesion to human epithelial cells, and reduces virulence in a murine model of systemic fungemia (Gale *et al.*, 1998), demonstrating that *INT1* contributes to the morphogenesis, adhesion, and virulence of *C. albicans*. However, Int1p is only one of many *C. albicans* adhesins (Cormack *et al.*, 1999; Staab *et al.*, 1999) and only one of several proteins that stimulate the morphologic switch

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TABLE 1
Strains and Plasmids Used

Strain name	Genotype	Source
yJB195	<i>Mat a-ura3-1 ade2-1 his3-11 leu 2-3,112 can 1-100 trp1-1</i>	Longtine <i>et al.</i> , 1993
yJB1845	<i>Mat a cdc12-6ts ade2 cry1 his4 leu2 trp1a ura3 SUP4-3ts</i>	Dr. Jamie Konopka
yJB3519	<i>Mat a leu2 ura 3 can1 ade2 his3 trp1 ssd1 ho Δcla4::URA3 Δclb1 Δclb3::TRP1 Δclb4::HIS3 Δbar1</i>	Tjandra <i>et al.</i> , 1998
yJB3560	<i>yJB4414, pCG110</i>	This work
yJB3561	<i>yJB195, pCG110</i>	This work
yJB3615	<i>Mat a ade1 trp1 leu2 his3 ura3 cdc1-1(ts)</i>	Paidhungat <i>et al.</i> , 1998
yJB3616	<i>yJB3615, pCG01</i>	This work
yJB3622	<i>yJB3621, pCG01</i>	This work
yJB4068	<i>Mat a ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, pBM272</i>	This work
yCG111	<i>Mat a ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, GAL10-INT1::URA3</i>	Dr. Cheryl Gale
Plasmid name	Relevant genes and markers	Source
pBM272	<i>pGAL10, URA3</i>	Johnston and Davis, 1984
pCG01	<i>pGAL10-INT1 in pBM272</i>	Gale <i>et al.</i> , 1960 C. Gale and J. Berman (unpublished)
pCG110	<i>pGAL10-INT1, LEU2</i>	This work
pMG1422	<i>pGAL10-INT1ΔEF 1-2 in pBM272</i>	
pJB1440 (pSWE1-29)	<i>pGAL10-SWE1, URA3</i>	Booher <i>et al.</i> , 1995

between yeast and filamentous growth (Braun *et al.*, 1997; Liu *et al.*, 1995). Interestingly, when *INT1* is expressed in *S. cerevisiae*, it is sufficient both to direct the adhesion of *S. cerevisiae* cells to human epithelial cells and to stimulate *S. cerevisiae* cells to form long, highly polarized buds reminiscent of *C. albicans* germ tubes (Gale *et al.*, 1998). Thus, expressing *INT1* in *S. cerevisiae* provides a unique opportunity to examine the mechanisms of *INT1*-induced filamentous growth (I-IFG) in a simpler yeast system.

Because the *Int1p* sequence includes at least two predicted divalent cation-binding motifs, we asked whether I-IFG in *S. cerevisiae* is dependent on the presence of divalent cations. We found that I-IFG is positively regulated by Mn^{2+} . Surprisingly, the regulation of I-IFG by Mn^{2+} was not eliminated by the deletion of the putative cation-binding motifs from the *INT1* coding sequence. Rather, we found that Mn^{2+} also regulates filament formation in these mutant backgrounds, indicating that polarized growth in *S. cerevisiae* is dependent upon the presence of Mn^{2+} ions.

MATERIALS AND METHODS

Strains, plasmids, and culture conditions. Strains and plasmids used in this study are listed in Table 1.

Strains were grown in standard laboratory salt dextrose complete (SDC) media with appropriate amino acid drop-outs (Sherman, 1991). Media were supplemented after autoclaving with a carbon source (as indicated in the text), 10 mM phosphate buffer (pH 6.6), EDTA, and salt solutions as indicated. Plasticware was used in all experiments.

To induce I-IFG, uninduced overnight cultures were diluted fivefold into medium containing 1% galactose and 1% raffinose (SC + gal/raf) to induce *pGAL-INT1* expression. Induced cultures were grown 16–24 h and then monitored for filamentation. Each experiment was done a minimum of three different times and a minimum of 100 cells was counted each time the experiment was done.

Construction of *pGAL-INT1-ΔEF1-2* (pMG1422). Plasmid pMG1400 contains *INT1* sequence from 18 nt upstream of the start codon to 870 bp downstream of the stop codon altered so that *PstI* restriction sites (underlined below) flank the two putative EF hand domains. These sites were generated by site-directed mutagenesis (Kunkel *et al.*, 1987) using oligonucleotides JB 463 (CACATTCTTACTATTGTTATCTGCAGTGGGACTAAGATATAAATTGAC) and JB464 (GTTCAAATATCTGATTTCTCAACTGCAGAAATATCATCATTATCTTC). Plasmid pMG1415 was generated by digestion of pMG1400 with *PstI* and religation of the plasmid lacking the 1050-bp fragment between the *PstI* sites. The *INT1* fragment in pMG1415 was digested with *BglII*

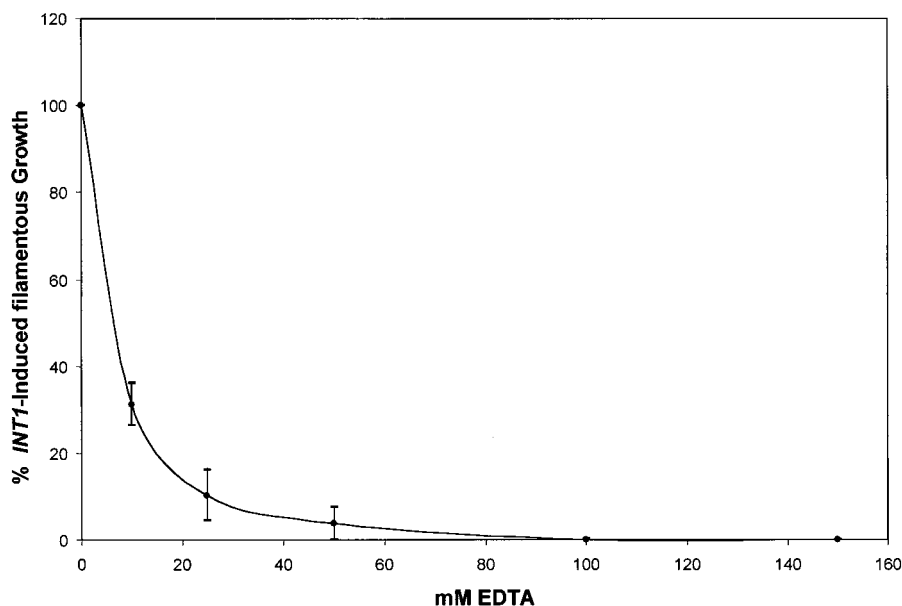


FIG. 1. Proportion of γ CG111 cells forming *INT1*-induced filaments in different concentrations of EDTA.

and *SaII* and inserted into pBM272 (Johnston and Davis, 1984) to generate pMG1422 (pGAL-*INT1*- Δ EF1-2).

Microscopy and image collection. Cell morphology was examined using a Nikon Eclipse E800 photomicroscope (Fryer Co., Huntley, IL) equipped with DIC optics, photographed using a 40X, 0.75 n.a. plan fluor objective and a CoolCam liquid-cooled, three-chip color CCD camera (Cool Camera Co. Decatur, GA), and captured with Image Pro Plus version 4.0 (Media Cybernetics, Silver Springs, MD).

Determination of nuclear DNA content. Cells induced to express *INT1* for 16 h were stained with mithromycin (Dien *et al.*, 1994) and analyzed by flow cytometry (Cytofluorograf IIs; Ortho Diagnostics) at 457 nm and 200 mW laser power, and green fluorescence was collected using a 515LP filter after passing through a short-pass dichroic beam splitter. Data were collected in list, area, and linear modes at rates of a few hundred cells per second using the Cicero data acquisition system and list-mode data was analyzed using CyCLOPS software (Cytomation Inc., Fort Collins, CO).

RESULTS AND DISCUSSION

EDTA reduces the formation and length of *INT1*-induced filaments. To determine whether divalent cat-

ion binding was important for I-IFG, we asked whether the divalent cation chelator EDTA affected the formation of Int1p-induced filaments. I-IFG was induced in strain γ CG111 (containing an integrated copy of *pGAL-INT1*). Cultures were incubated 16 h at 30°C, and the proportion of filamentous cells in the population was recorded. With increasing EDTA concentration, there was a corresponding decrease in the proportion of filamentous cells (Fig. 1). Furthermore, the few filaments that did form in the presence of EDTA were much shorter than those formed in the absence of EDTA (Fig. 2B).

Cells exposed to EDTA appeared to be arrested, in that they formed few buds or filaments. To determine whether exposure to EDTA simply caused growth arrest or whether it affected long-term cell viability, cells exposed to EDTA were returned to fresh, chelator-free glucose-containing medium (to repress *pGAL-INT1*) and the number of colony forming units (cfu) was compared for EDTA-treated versus untreated cells. The number of cfu relative to the untreated control was not significantly reduced with increasing EDTA concentration (up to 150 mM EDTA, data not shown), indicating that the EDTA-mediated decrease in I-IFG was not due to cell death.

The addition of Mn^{2+} to EDTA-treated cells restores I-IFG. To determine whether a specific divalent cation was limiting in EDTA-treated cultures, we asked whether Mg^{2+} , Ca^{2+} , or Mn^{2+} (each at 1, 10, and 20 mM) addition could counteract the EDTA inhibition of I-IFG.

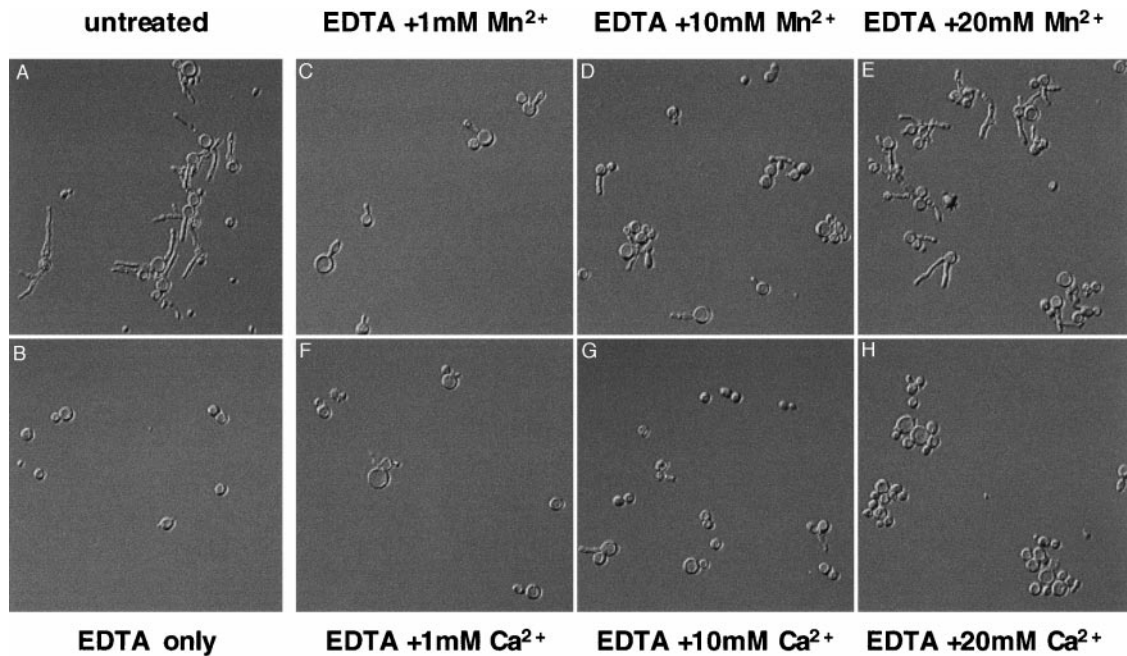


FIG. 2. Micrographs of yCG111 cells grown in galactose/raffinose medium only (A), and including 20 mM EDTA plus Mn^{2+} or $CaCl_2$ as indicated (B–H).

Interestingly, the addition of Mn^{2+} to cultures containing EDTA completely restored I-IFG: both the percentage of cells forming filaments and the average filament length

were close to the levels of I-IFG seen in the cultures prior to the addition of EDTA (Fig. 2 and Fig. 3). In contrast, addition of Ca^{2+} or Mg^{2+} restored growth, in that budding

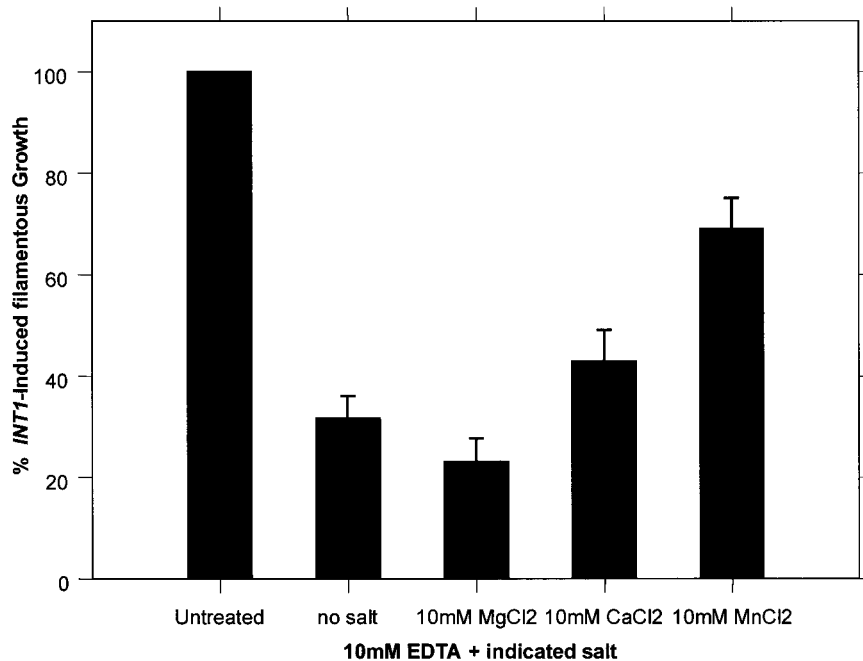


FIG. 3. The proportion of yCG111 cells forming *INT1*-induced filaments in the indicated concentrations of EDTA and divalent cations.

cells were observed, but neither Ca^{2+} nor Mg^{2+} restored filament formation to the same degree as did the addition of Mn^{2+} (Figs. 2 and 3). In addition, with increasing Mn^{2+} concentration, there was a corresponding increase in the proportion of filamentous cells (Figs. 2C–2E), suggesting a direct relationship between the Mn^{2+} concentration and the degree of filamentation. In contrast, no relationship between Ca^{2+} or Mg^{2+} concentration and the proportion of filamentous cells was observed. Even at the highest Ca^{2+} concentrations used, the rare filaments that did appear were very short and resembled those seen in EDTA-treated cultures (Figs. 2F–2H). Thus, Mn^{2+} appears to have a specific role in filamentous growth that cannot be provided by either Mg^{2+} or Ca^{2+} .

Because Mn^{2+} has a higher affinity for EDTA than does Ca^{2+} or Mg^{2+} ($\log K = 14.0, 10.7, 8.7$, respectively), it was possible that Mn^{2+} simply titrated the chelator more efficiently and thus liberated another cation required for I-IFG. To test this hypothesis, we asked whether Ni^{2+} , which has a much higher affinity for EDTA ($\log K = 18.6$) than Mn^{2+} , could restore I-IFG in the presence of EDTA. The addition of 20 mM Ni^{2+} to 20 mM EDTA-treated culture did not restore I-IFG any more than did the addition of Ca^{2+} (data not shown). Thus, the response of I-IFG to Mn^{2+} cannot be attributed to a simple liberation of ions chelated by EDTA. Rather, our observations are consistent with the hypothesis that the Mn^{2+} ion concentration is important for an aspect of I-IFG that affects both the proportion of cells producing filaments and the length of the filaments formed.

Mn^{2+} ions do not affect the EDTA-induced cell cycle delay. I-IFG requires that cells traverse the cell cycle and be able to initiate daughter bud emergence (C. Gale and J. Berman, unpublished results).

Loukin and Kung (1995) previously reported that treatment with divalent cation chelator BAPTA delays the cell cycle progression of *S. cerevisiae* cells (Loukin and Kung, 1995; Paidhungat and Garrett, 1998). To determine whether the ability of Mn^{2+} to restore I-IFG was due to Mn^{2+} ameliorating the EDTA effect on the cell cycle, we compared the DNA content of cells treated with EDTA to the DNA content of cells treated with EDTA and $MnCl_2$ using flow cytometry. Cells expressing *pGAL-INT1* (YCG111) and a control strain containing the *pGAL* without the *INT1* insert (YJB4068) were cultured overnight in SC + gal/raf medium containing EDTA alone or EDTA with Mn^{2+} and then stained with mithromycin and analyzed by flow cytometry. Because the filaments formed in *INT1*-expressing cells interfere with flow cytometric analysis of the cells, we monitored the cell cycle effects on the

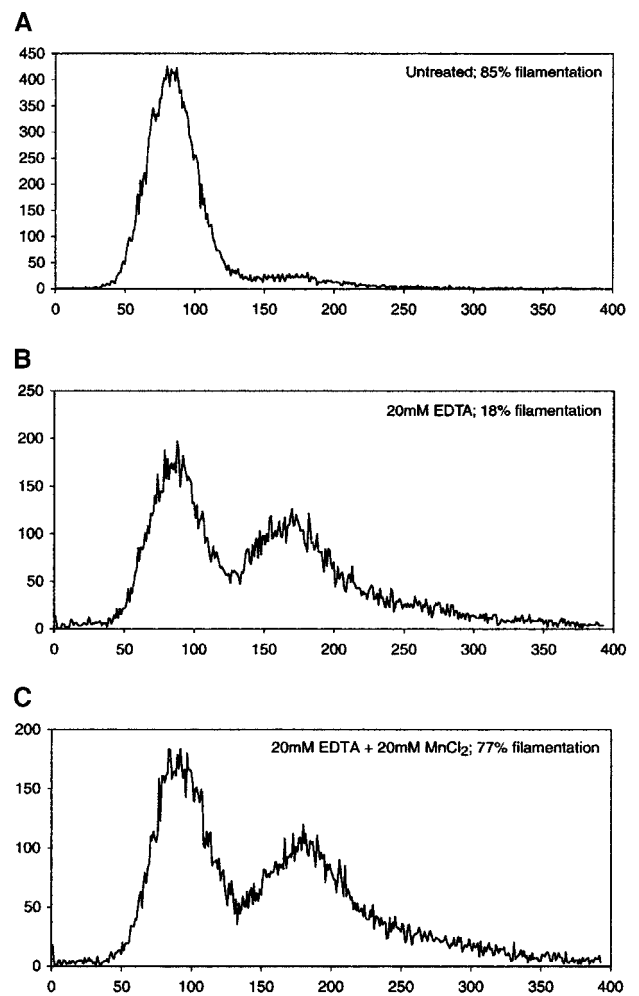


FIG. 4. DNA content of yJB4068 cells grown in galactose/raffinose medium with EDTA and $MnCl_2$ as indicated and analyzed by flow cytometry.

control strain and analyzed the extent of I-IFG in cells expressing *Int1p* and grown under identical conditions. The *Int1p*-expressing strain (yCG111) and the control strain (yJB4068) were grown in SC + gal/raf. Because galactose and raffinose are poor carbon sources, these cultures grew slowly and most cells in the population remained in G1, with 1N DNA content (Fig. 4A). In the parallel *INT1*-expressing culture, 85% of the cells formed filaments.

Cells grown in galactose/raffinose plus 20 mM EDTA displayed an increase in the proportion of cells with 2N DNA content; under the same growth conditions, the parallel yCG111 culture produced only 18% filaments (Fig. 4B). Thus, as was observed for cells grown in glucose and BAPTA (Loukin and Kung, 1995), cells grown in

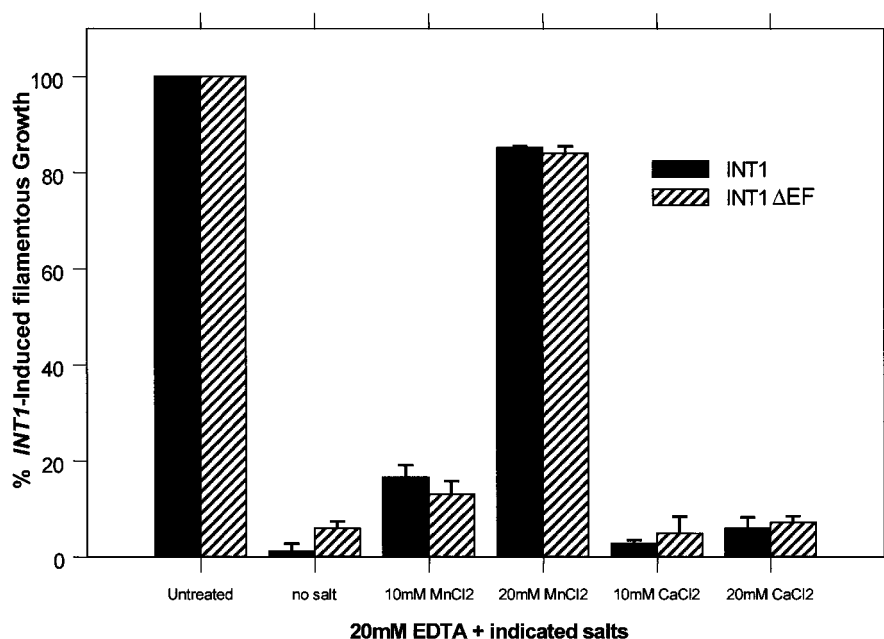


FIG. 5. Proportion of cells expressing pGAL-*INT1* (pCG01) or pGAL-*INT1-ΔEF1-2* (pMG1422) that formed filaments in the indicated concentrations of EDTA and divalent cations.

SC + gal/raf and EDTA exhibit a cell cycle delay. Importantly, when cells were grown in SC + gal/raf including 20 mM EDTA and 20 mM Mn²⁺ (Fig. 4C), the number of 2N cells was similar to that seen in the presence of SC + gal/raf and 20 mM EDTA alone, indicating that the addition of Mn²⁺ to EDTA-treated cells did not alter the cell cycle distribution relative to cells incubated with EDTA alone. Yet the addition of Mn²⁺ resulted in a more than fourfold increase in the proportion of exhibiting I-IFG relative to the EDTA-only culture. Thus, Mn²⁺ does not ameliorate the EDTA effect on I-IFG by altering the effect of EDTA on cell cycle progression.

Reducing the intracellular Mn²⁺ concentration with *smf1* or *cdc1* mutations does not affect filamentous growth. To determine whether EDTA inhibits filamentation by reducing the intracellular divalent cation levels, we examined the ability of mutants with reduced intracellular Mn²⁺ concentration to form filaments. *S. cerevisiae* genes *SMF1* and *CDC1* affect cytoplasmic Mn²⁺ homeostasis. *SMF1* encodes the *S. cerevisiae* high-affinity manganese transporter (Supek *et al.*, 1996), and *CDC1* appears to play a role in the maintenance of cytosolic manganese levels (Loukin and Kung, 1995; Paidhungat and Garrett, 1998). Mutation of both genes is synthetically lethal. Strains carrying an *smf1* null allele or a *cdc1^{ts}* allele and corresponding isogenic wild-type control strains were transformed with

pCG01. *INT1* expression was induced by growth on SC + gal/raf at 23°C (the semipermissive temperature for the *cdc1^{ts}* allele). For all of these strains, the proportion of filaments in the congenic mutant and wild-type strain pairs was not different under all growth conditions tested (data not shown). Because *smf1* and *cdc1* mutations both reduce the intracellular concentration of Mn²⁺ in yeast, these results imply that I-IFG is not dependent upon wild-type levels of intracellular Mn²⁺. Instead, these results are consistent with the hypothesis that external Mn²⁺ ions are critical for I-IFG.

Deletion of *INT1* EF-hand and MIDAS motifs does not eliminate the Mn²⁺ effect on I-IFG. The putative EF-hands and MIDAS domains (Gale *et al.*, 1996) are predicted to be in the extracellular portion of the protein (C. Bendel and M. Hostetter, unpublished results). We asked whether I-IFG was dependent on these putative domains in Int1p. A deletion derivative of pGAL-*INT1* that removed both EF-hands and the putative MIDAS domain between them, pGAL-*INT1Δ*-EF1-2 (pMG1422), was expressed in *S. cerevisiae*. We expected that if extracellular Mn²⁺ was bound by Int1p to mediate I-IFG then I-IFG would be eliminated by the deletion of these divalent cation-binding domains. Surprisingly, the Int1-*Δ*EF1-2 protein retained the ability to induce a high proportion of filaments that were similar in length to those induced by full-length Int1p. Interestingly, the proportion

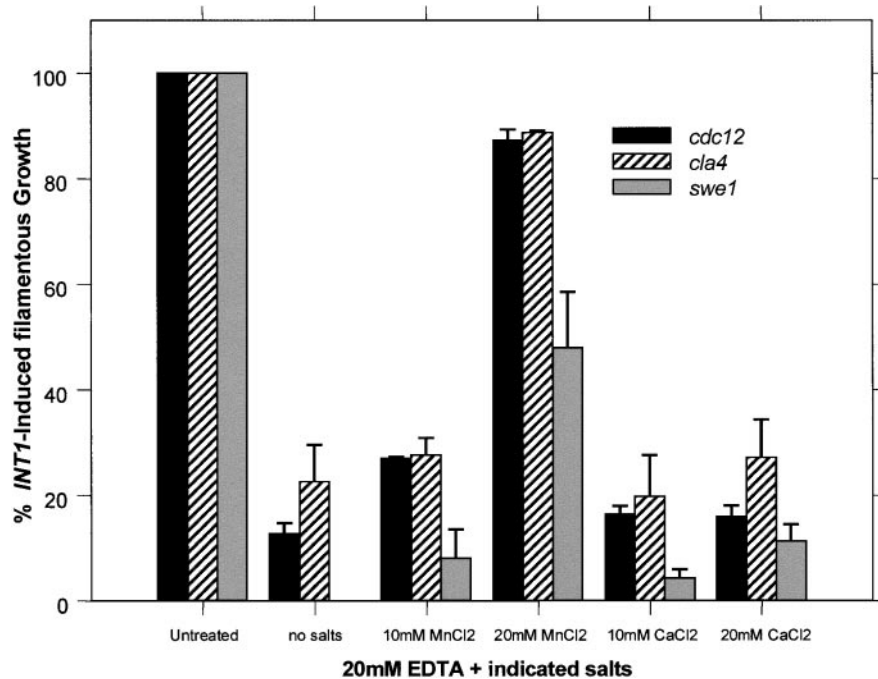


FIG. 6. The proportion of *cdc12-6^{ts}* (yJB184501), *cla4*, *clb1*, *clb3*, and *clb4* (yJB3519) and Swe1p-overexpressing (yJB3046 + p1440) cells forming filaments in the indicated concentrations of EDTA and divalent cations.

and length of filaments induced by pGAL-*INT1* and pGAL-*INT1-ΔEF1-2* were very similar and the filamentation in response to addition of EDTA, EDTA and Mn^{2+} , and EDTA and Ca^{2+} was also similar for the two plasmids (Fig. 5). Thus, the Mn^{2+} dependence of I-IFG is not due to interaction of Mn^{2+} ions with the putative EF-hand or MIDAS domains of Int1p. This result suggests that either Mn^{2+} ions affect a different domain of Int1p or I-IFG is regulated by some other Int1p-independent, Mn^{2+} -dependent process.

Mn²⁺ concentration is important for other types of filamentous growth. To determine whether this dependence on Mn^{2+} concentration is unique to I-IFG or is a general property of filamentous growth in *S. cerevisiae*, we examined the effects of EDTA and Mn^{2+} on three different *S. cerevisiae* genotypes that result in highly polarized growth. Mutation of *CDC12* disrupts the formation of septins at the bud neck and results in the growth of elongated buds at the permissive temperature (Longtine *et al.*, 1996), a *cla4 clb1 clb3 clb4* strain exhibits hyperpolarized growth (Tjandra *et al.*, 1998), and a strain overexpressing *SWE1*, the morphogenesis checkpoint gene, is delayed in G2/M and forms hyperpolarized buds (Mizunuma *et al.*, 1998; Lew and Reed, 1995). Upon addition of 20 mM EDTA to cultures of

these three strains, the proportion of cells forming filaments was reduced significantly. Furthermore, the degree of filamentation was restored upon the addition of Mn^{2+} , but was not restored when Ca^{2+} was added (Fig. 6). Therefore, the concentration of Mn^{2+} plays an important role in the regulation of multiple types of polarized growth in *S. cerevisiae*. Whereas multiple *S. cerevisiae* proteins may be required for the cation dependence of I-IFG, the current experiments cannot rule out the possibility that Int1p, like vertebrate integrins, also binds divalent cations directly. Perhaps the divalent cation-binding domains of Int1p, as in vertebrate integrins (Dickeson *et al.*, 1997; Dransfield *et al.*, 1992; McDowall *et al.*, 1998; Michishita *et al.*, 1993; Mould *et al.*, 1995), mediate the adhesion function of Int1p rather than the morphogenetic function of Int1p.

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REFERENCES

- Braun, B. R., and Johnson, A. D. 1997. Control of filament formation in *Candida albicans* by the transcriptional repressor *TUP1*. *Science* **277**: 105–109.
- Cormack, B. P., Ghori, N., and Falkow, S. 1999. An adhesin of the yeast pathogen *Candida glabrata* mediating adherence to human epithelial cells. *Science* **285**: 578–582.
- Corner, B. E., and Magee, P. T. 1997. *Candida* pathogenesis: Unraveling the threads of infection. *Curr. Biol.* **7**: R691–R694.
- Dickeson, S. K., Walsh, J. J., and Santoro, S. A. 1997. Contributions of the I and EF hand domains to the divalent cation dependent collagen binding activity of the $\alpha 2\beta 1$ integrin. *J. Biol. Chem.* **272**: 7661–7668.
- Dien, B. S., Peterson, M. S., and Srienc, F. 1994. Cell-cycle analysis of *Saccharomyces cerevisiae*. *Methods Cell Biol.* **42**: 457–475.
- Dransfield, I., Cabanas, C., Craig, A., and Hogg, N. 1992. Divalent cation regulation of the function of the leukocyte integrin LFA-1. *J. Cell Biol.* **116**: 219–226.
- Gale, C., Finkel, D., Tao, N., Meinke, M., McClellan, M., Olson, J., Kendrick, K., and Hostetter, M. 1996. Cloning and expression of a gene encoding an integrin-like protein in *C. albicans*. *Proc. Natl. Acad. Sci. USA* **93**: 357–361.
- Gale, C., Bendel, C. M., McClellan, M., Hauer, M., Becker, J. M., Berman, J., and Hostetter, M. K. 1998. Linkage of adhesion, filamentous growth, and virulence in *C. albicans* to a single gene, *INT1*. *Science* **279**: 1355–1358.
- Johnston, M., and Davis, R. 1984. Sequences that regulate the divergent GAL1-GAL10 promoter in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **4**: 1440–1448.
- Kunkel, T. A., Roberts, J. D., and Zalkour, R. A. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* **154**: 367–382.
- Lew, D. J., and Reed, S. I. 1995. A cell cycle checkpoint monitors cell morphogenesis in budding yeast. *J. Cell Biol.* **129**: 739–749.
- Liu, H., Kohler, J., and Fink, G. R. 1995. Suppression of hyphal formation in *Candida albicans* by mutation of a *STE12* homolog. *Science* **266**: 1723–1726.
- Lo, H. J., Kohler, J. R., DiDomenico, B., Loebenberg, D., Cacciapuoti, A., and Fink, G. R. 1997. Nonfilamentous *C. albicans* mutants are avirulent. *Cell* **90**: 939–949.
- Longtine, M. S., DeMarini, D. J., Valencik, M. L., Al-Awar, O. S., Fares, H., DeVirgilio, C., and Pringle, J. R. 1996. The septins: Role in cytokinesis and other processes. *Curr. Opin. Cell Biol.* **8**: 106–119.
- Loukin, S., and Kung, C. 1995. Manganese effectively supports yeast cell-cycle progression in place of calcium. *J. Cell Biol.* **131**: 1025–1037.
- McDowall, A., Leitinger, B., Stanley, P., Bates, P. A., Randi, A. M., and Hogg, N. 1998. The I-domain of integrin leukocyte function-associated antigen-1 is involved in a conformational change leading to high affinity binding to ligand intercellular adhesion molecule 1 (ICAM-1). *J. Biol. Chem.* **273**: 27396–27403.
- Michishita, M., Videm, V., and Arnaout, M. 1993. A novel divalent cation-binding site in the A domain of the $\beta 2$ integrin CR3 (Cd11b/CD18) is essential for ligand binding. *Cell* **72**: 857–867.
- Mitchell, A. P. 1998. Dimorphism and virulence in *Candida albicans*. *Curr. Opin. Microbiol.* **1**: 687–692.
- Mizunuma, M., Hirata, D., Miyshara, K., Tsuchiya, E., and Miyakawa, T. 1998. Role of calcineurin and Mpk1 in regulating the onset of mitosis in budding yeast. *Nature* **392**: 303–306.
- Mould, A. P., Akiyama, S. K., and Humphries, M. J. 1995. Regulation of integrin $\alpha 5\beta 1$ -fibronectin interactions by divalent cations. *J. Biol. Chem.* **270**: 26270–26277.
- Odds, F. C. 1988. *Candida and Candidosis*. Tindall, London.
- Paidhungat, M., and Garrett, S. 1998. *CDC1* is required for growth and Mn^{2+} regulation in *Saccharomyces cerevisiae*. *Genetics* **148**: 1777–1786.
- Sherman, F. 1991. Getting started with yeast. *Methods Enzymol.* **194**: 3–21.
- Stabb, J. F., Bradway, S. D., Fidel, P. L., and Sundstrom, P. 1999. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science* **283**: 1535–1538.
- Supek, F., Supekova, L., Nelson, H., and Nelson, N. 1996. A yeast manganese transporter related to the macrophage protein involved in conferring resistance to mycobacteria. *Proc. Natl. Acad. Sci. USA* **93**: 5105–5110.
- Tjandra, H., Compton, J., and Kellogg, D. 1998. Control of mitotic events by the Cdc42 GTPase, the Clb2 cyclin and a member of the PAK kinase family. *Curr. Biol.* **24**: 991–1000.