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Communication

Effect of Ethanol on Cell Growth of Budding Yeast: Genes That Are Important for Cell Growth in the Presence of Ethanol

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The budding yeast *Saccharomyces cerevisiae* has been used in the fermentation of various kinds of alcoholic beverages. But the effect of ethanol on the cell growth of this yeast is poorly understood. This study shows that the addition of ethanol causes a cell-cycle delay associated with a transient dispersion of F-actin cytoskeleton, resulting in an increase in cell size. We found that the tyrosine kinase Swe1, the negative regulator of Cdc28-Clb kinase, is related to the regulation of cell growth in the presence of ethanol. Indeed, the increase in cell size due to ethanol was partially abolished in the *SWE1*-deleted cells, and the amount of Swe1 protein increased transiently in the presence of ethanol. These results indicated that Swe1 is involved in cell size control in the presence of ethanol, and that a signal produced by ethanol causes a transient up-regulation of Swe1. Further we investigated comprehensively the ethanol-sensitive strains in the complete set of 4847 non-essential gene deletions and identified at least 256 genes that are important for cell growth in the presence of ethanol.

Key words: actin; cell-cycle; cell-size; ethanol; Swe1

The budding yeast *Saccharomyces cerevisiae* has been used in the fermentation of various kinds of alcoholic beverages. Although ethanol is a final product of anaerobic fermentation of sugars by yeast, it is toxic to yeast cells and induces stress responses such as the expression of heat shock proteins and the accumulation of trehalose.[1)] But the effect of ethanol on the cell growth, cell cycle, and morphogenesis of budding yeast is unclear.

To investigate the effect of ethanol on the cell growth of yeast, we used the *S. cerevisiae* laboratory strain W303 (*MATa trp1 leu2 ade2 ura3 his3 can1-100*, from Rothstein). First we examined the effect of various concentrations of ethanol on the growth of wild-type cell culture (WT) of the early-log phase ($1–2 \times 10^6$ cells/ml). A 6% concentration of ethanol decreased the growth rate of the cells by 50% (data not shown). By using this concentration of ethanol, we examined the effect of ethanol on cell growth. Cell growth was inhibited for 2 h after the addition of ethanol and resumed after 3 h (WT in Fig. 1A). To investigate the details of the growth inhibition, we examined cell cycle progression, morphology, and cell size in the presence of ethanol. The FACS (flow-cytometry analysis) profile of the culture showed that the addition of ethanol causes a decrease in cells of S-phase, the boundary region between 1C and 2C (see the profile at 1 h in 6% EtOH in Fig. 1B). The populations of cells with unbud, small bud, or large bud changed little in the presence of ethanol (Fig. 1C). The cell size of mother cells increased (Fig. 1D). These results suggested that transient growth inhibition due to ethanol result from a delay in the interphase of the cell cycle.

To investigate the effect of ethanol on actin cytoskeleton, we examined the distribution of F-actin in WT cells in the presence of ethanol. In the absence of ethanol, F-actin is localized as patches at the polarized growing regions, bud site, and bud neck, and actin cables were observed too (Fig. 2A, 0 min). But F-actin at the polarized growing regions was dispersed throughout the cells within 10 min after the addition of ethanol, and actin cables were disrupted by the addition of ethanol. Dispersion of F-actin was observed until at least 60 min after the addition of ethanol (Fig. 2A), and F-actin was re-localized to the polarized growing regions at the restart of growth (data not shown). This result indicated that the spatial organization of the actin cytoskeleton is transiently disrupted by the addition of ethanol.

Since the above results indicated that ethanol induces a delay in the cell cycle, we investigated whether the

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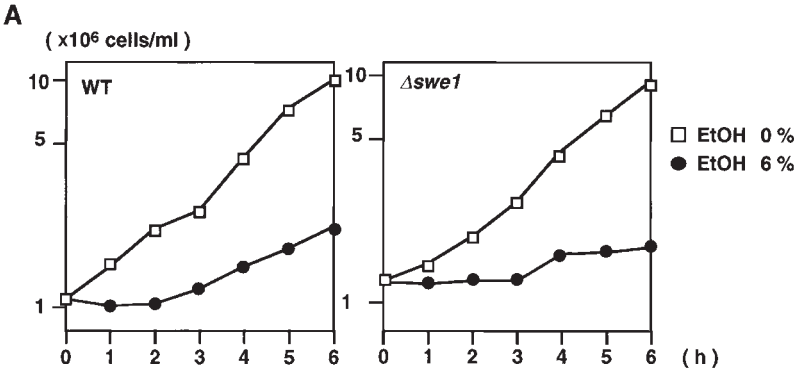

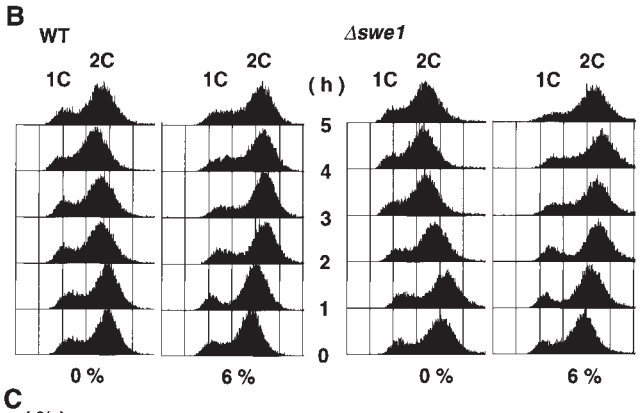

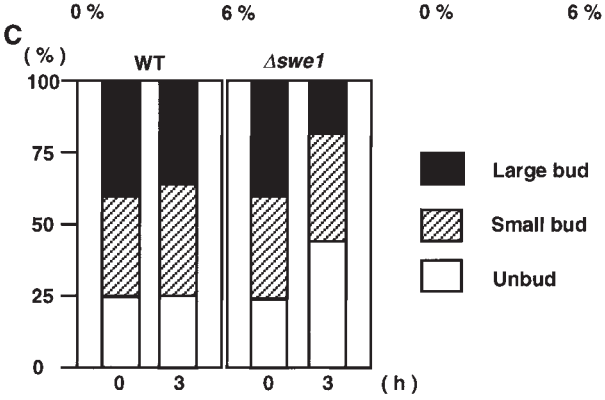

D

(h)	(μm) WT L (%)	WT S (%)	Δswe1 L (%)	Δswe1 S (%)
0	6.56 ± 1.04 (100)	4.69 ± 0.70 (100)	5.73 ± 0.77 (100)	4.68 ± 0.58 (100)
3	6.66 ± 0.75 (101.5)	5.86 ± 0.78 (124.9)	6.08 ± 0.95 (106.1)	5.02 ± 0.88 (107.3)

Fig. 1. Effect of Ethanol on Cell Growth of Wild-type and Δ*swe1* Cells.

A-D: Ethanol (final concentration 6%) was added to cultures of wild-type (WT, W303/DHT22-1B) and Δ*swe1* cells grown to early-log phase (1–2 × 10^6 cells/ml) in YPD medium at 25 °C. The cells taken at the times indicated were used for measurement of cell growth (A), FACS analysis[2] (B), morphology (C), and size of mother cells (D). Cell growth (A) was measured by Sysmex F-820 (SYSMEX CO.). Cell Morphology (C) was classified into three groups: large bud (bud size approximately up to 25% of mother cell), small bud (bud size less than 25% of mother cell), and unbud cells. The long axis (L) and short axis (S) of the mother cells were measured in D.

regulator(s) of cell cycle progression are important for cell growth in the presence of ethanol. We examined the ethanol-sensitivities of various mutants that are defective in a negative regulator of Cdc28-Clb kinase (*SWE1*), a negative regulator of the *SWE1* transcription (*ZDS1*), regulators of protein-degradation (*MET30*, *CDC4*, and *CDC23*), signal transduction pathways (*CNB1*, *MPK1*, *HOG1*, *MCK1/SCZ10*, *PKC1*), transcription factors (*SWI4* and *SWI6*), and microtubule-regulating factor (*BIM1*). Among these mutants, nine

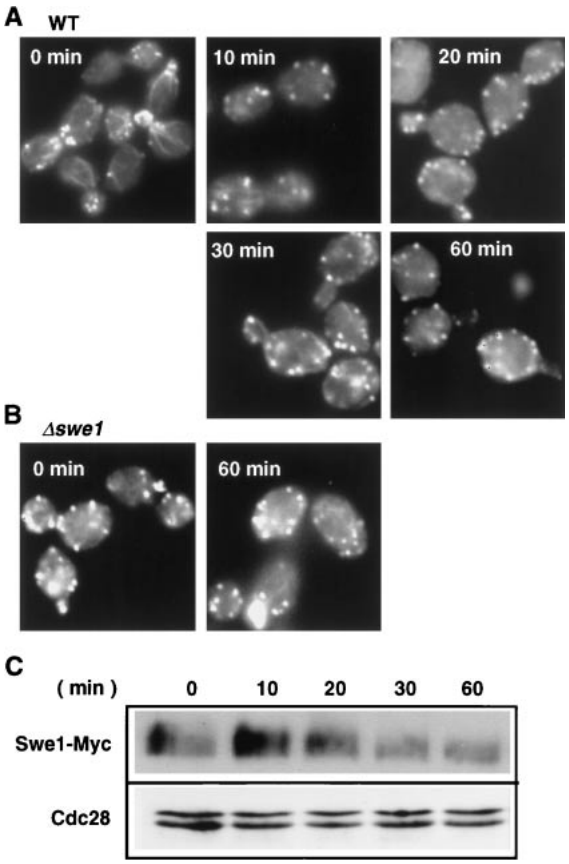

Fig. 2. Effect of Ethanol on Actin Cytoskeleton and Swe1 Protein.
A, B: Ethanol (final concentration 6%) was added to cultures of WT (W303) (A) and Δ*swe1* cells (B) grown to early-log phase ($1–2 \times 10^6$ cells/ml) in YPD medium at 25 °C. The actin cytoskeleton in the cells taken at the times indicated was stained with rhodamine-phalloidine.[3] C: Ethanol (final concentration 6%) was added to cultures of WT having the Myc epitope-tagged *SWE1* gene[3] grown to early-log phase ($1–2 \times 10^6$ cells/ml) in YPD medium at 25 °C. The cells were taken at the times indicated. Total proteins were run on SDS-polyacrylamide gels. For detection of Myc-tagged Swe1 or Cdc28, monoclonal antibodies 9E10 (BAbCO) to the Myc epitope or anti-PSTAIR (Santa Cruz Biotech), respectively, was used.[4]

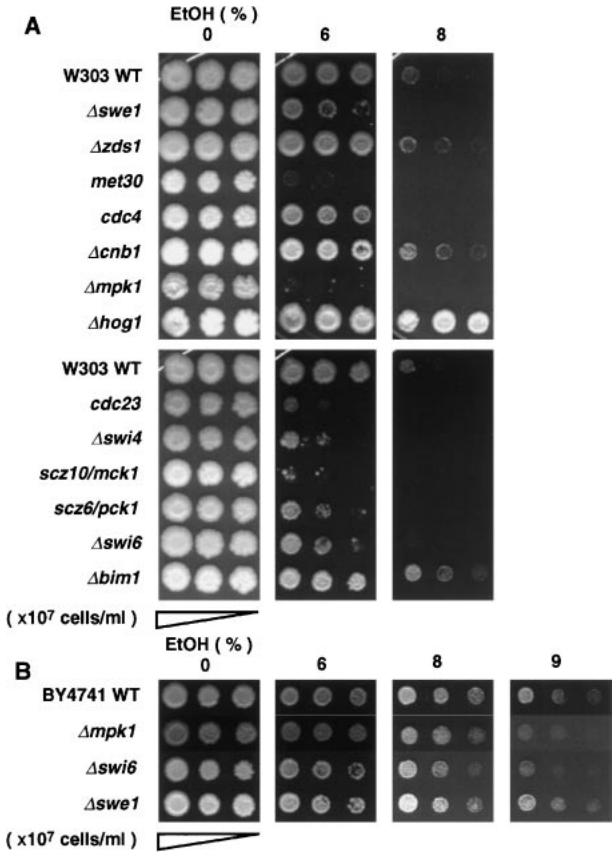

Fig. 3. Ethanol Sensitivities of Various Mutants.
A: The indicated mutants of the W303 strain freshly cultured on YPD solid medium were serially diluted ($10–1 \times 10^7$ cells/ml), spotted onto YPD solid medium containing the indicated concentrations of ethanol, and cultured at 28 °C for 3 days. B: The ethanol sensitivities of the indicated mutants of the BY4741 strain were examined as in A.

mutants, Δ*swe1*, *met30*, *cdc4*, Δ*mpk1*, *cdc23*, Δ*swi4*, *mck1*, *pkc1*, and Δ*swi6*, exhibited ethanol sensitivity (Fig. 3), indicating that these genes are important for cell growth in the presence of ethanol. We focused on the role of Swe1 in the presence of ethanol, because ethanol induces a delay in the inter-phase of the cell cycle and Swe1 negatively regulates the onset of mitosis by inhibiting Cdc28-Clb kinase.[5]

To investigate the role of Swe1 in cell growth in the presence of ethanol, we examined cell number, cell cycle progression, morphology, and cell size of the *SWE1*-deleted cells (Δ*swe1*) after the addition of 6% ethanol. A delay in cell growth in the Δ*swe1* cells was observed as in the WT cells (Fig. 1A). Furthermore, in the Δ*swe1* cells, a decrease in cells of S-phase was observed as in WT (see the profile at 1 h in 6% EtOH in Fig. 1B), and unbud cells increased and cells with large bud decreased (Fig. 1C). These results indicated that an inhibition of the G1-S transition but not of the G2-M transition occurs in the Δ*swe1* cells. Consistent with this result, the increase in cell size of the Δ*swe1* cells was less than that of the WT cells (Fig. 1D), indicating that Swe1 is involved in cell size control in the presence of ethanol. A dispersion of F-actin after the addition of ethanol in the Δ*swe1* cells was observed as in the WT cells (Fig. 2B).

Swe1 negatively regulates the onset of mitosis by inhibiting Cdc28-Clb kinase.[5] It is possible that Swe1 is up-regulated by the addition of ethanol. To investigate this, we examined the level of the Swe1 protein in the presence of ethanol. As shown in Fig. 2C, the Swe1 protein level increased within 10 min after addition of ethanol. After 20 min, the Swe1 protein level decreased to the basal level (at time 0). Swe1 is targeted for rapid degradation in G2/M during the cell cycle.[6] A signal produced by ethanol might transiently inhibit the ubiqutin-proteasome system for Swe1-degradation.

To identify the genes that are important for cell growth in the presence of ethanol, we investigated

Table 1. Genes That Are Important for Cell Growth in the Presence of 11% Ethanol

Classification (No. of genes)	Gene name/ORF
Biosynthesis (43)	ADH1/YOL086C ADO1/YJR105W ALG6/YOR002W ATP1/YML022W ARC1/YGL105W ARD1/YHR013C ARO1/YDR127W ARO7/YPR060C BNA1/YJR025C CTR1/YPR124W DIA4/YHR011W ERG2/YMR202W ERG28/YER044C FEN1/YCR034W FRS1/YLR060W GPH1/YPR160W HSC82/YMR186W MNN11/YJL183W MRPS17/YMR188C MSM1/YGR171C MSR1/YHR091C NAT3/YPR131C PRO1/YDR300C PRP18/YGR006W PRS5/YOL061W RIB1/YBL033C RIB4/YOL143C RML2/YEL050C RPL7A/YGL076C RPL13B/YMR142C RPL39/YJL189W RPP1A/YDL081C RPS1B/YML063W RPS10B/YMR230W RPS16A/YMR143W SPF1/YEL031W SUR4/YLR372W TPS1/YBR126C TPS2/YDR074W TRP1/YDR007W TRP2/YER090W TRP3/YKL211C TRP4/YDR354W
Cell cycle (17)	BUB1/YGR188C BUR2/YLR226W CDC40/YDR364C CDH1/YGL003C CLN3/YAL040C CTF18/YMR078C DOC1/YGL240W GRR1/YJR090C HTL1/YCR020W MEC3/YLR288C PHO85/YPL031C RNR4/YGR180C SIT4/YDL047W SNT1/YCR033W SWI4/YER111C SWI6/YLR182W SWM1/YDR260C
Cytoskeleton (18)	ALF1/YNL148C BIK1/YCL029C CIK1/YMR198W CIN1/YOR349W CIN2/YPL241C CIN8/YEL061C CNM67/YNL225C END3/YNL084C GIM3/YNL153C GIM4/YEL003W GIM5/YML094W KAR3/YPR141C KIP2/YPL155C PAC10/YGR078C PFD1/YJL179W RVS161/YCR009C TPM1/YNL079C YKE2/YLR200W
Mitochondria (22)	ABF2/YMR072W ADK1/YDR226W ATP10/YLR393W ATP11/YNL315C ATP12/YJL180C CYC3/YAL039C GRX5/YPL059W MDJ1/YFL016C MDM10/YAL010C MDM34/YGL219C MGM101/YJR144W MRPL7/YDR237W MSH1/YHR120W RIP1/YEL024W RPO41/YFL036W SOD2/YHR008C TCM62/YBR044C TIM13/YGR181W TOM5/YPR133W-A TOM7/YNL070W TOM37/YMR060C TOM70/YNL121C
Morphogenesis (14)	BEM1/YBR200W BEN2/YER155C BNI1/YNL271C BUD19/YJL188C BUD25/YER014C-A BUD27/YFL023W BUD32/YGR262C CWH36/YCL007C MNN10/YDR245W OCH1/YGL038C RHO4/YKR055W SMI1/YGR229C SSD1/YDR293C TPD3/YAL016W
Nucleic acid binding (12)	DBP7/YKR024C EST1/YLR233C GCN5/YGR252W HRP1/YDR138W NPL3/YDR432W NSR1/YGR159C POL32/YJR043C RAD27/YKL113C REF2/YDR195W RSC1/YGR056W SPT10/YJL127C ZUO1/YGR285C
Protease (4)	AFG3/YER017C DOA4/YDR069C KEX2/YNL238W RPN4/YDL020C
Protein transport/Vacuole (45)	CCZ1/YBR131W CHC1/YGL206C CLC1/YGR167W COG1/YGL223C CUP5/YEL027W DID4/YKL002W DRS2/YAL026C GCS1/YDL226C GLO3/YER122C LUV1/YDR027C OPT2/YPR194C PEP3/YLR148W RAV1/YJR033C RIC1/YLR039C SAC2/YDR484W SEC28/YIL076W SEC66/YBR171W SNF8/YPL002C STP22/YCL008C TFP1/YDL185W TRS33/YOR115C VAC8/YEL013W VAM7/YGL212W VMA6/YLR447C VMA8/YEL051W VMA13/YPR036W VMA21/YGR105W VMA22/VHR060W VPH1/YOR270C VPH2/YKL119C VPS4/YPR173C VPS15/YBR097W VPS16/YPL045W VPS20/YMR077C VPS25/YJR102C VPS28/YPL065W VPS33/YLR396C VPS34/YLR240W VPS36/YLR417W VPS45/YGL095C VPS63/YLR261C VPS65/YLR322W VPS71/YML041C VPS72/YDR485C YPT6/YLR262C
Signal transduction (4)	IRA2/YOL081W PLC1/YPL268W RAS2/YNL098C SIP5/YMR140W
Transcription (25)	BDF1/YLR399C CTK2/YJL006C CTK3/YML112W CYC8/YBR112C DEP1/YAL013W DST1/YGL043W ELP2/YGR200C GCR2/YNL199C MKS1/YNL076W MOT2/YER068W NGG1/YDR176W NOT5/YPR072W ROX3/YBL093C RPA12/YJR063W RPB4/YJL140W RPB9/YGL070C RTG1/YOL067C RTG2/YGL252C SNF6/YHL025W SPT20/YOL148C SPT21/YMR179W SRB2/YHR041C SRB5/YGR104C THP2/YHR167W UME6/YDR207C
Transporter (11)	BRO1/YPL084W CSG2/YBR036C DAL5/YJR152W FUR4/YBR021W GTR1/YML121W ISA1/YLL027W ISA2/YPR067W NHA1/YLR138W NUP84/YDL116W NUP133/YKR082W PMR1/YGL167C
Unknown (41)	BRE5/YNR051C CAF17/YJR122W EMI1/YDR512C GON7/YJL184W HUR1/YGL168W LDB7/YBL006C NPL6/YMR091C RIM9/YMR063W RMD7/YER083C SET6/YPL165C YAF9/YNL107W YAL037W YBL094C YBR077C YDL099W YDR049W YDR114C YDR532C YEL059W YFR011C YGL218W YJR011C YJR018W YKL037W YKL118W YKR007W YKR035C YLR091W YLR358C YLR414C YML094C-A YMR157C YMR326C YNL056W YNL119W YNL120C YNL171C YNR025C YOR331C YPL144W YPL260W

The underlined genes are important for cell growth in the presence of 8% ethanol.

comprehensively the ethanol-sensitive strains in the complete set of 4,847 non-essential gene deletions. Since the genetic background of strain BY4741 (*MATa his3-1 leu2-2 met5-0 ura3-0*, from Invitrogen) used in the deletion set is distinct from that of strain W303, we first examined the ethanol sensitivity of the BY4741 deletion mutants, Δ*mpk1*, Δ*swi6*, and Δ*swe1*, which showed ethanol-sensitivity in W303 background. The BY4741 WT cells were more resistant to ethanol than the W303 WT cells (Fig. 3). The Δ*mpk1* and the Δ*swi6* but not the Δ*swe1* in the BY4741 strain showed ethanol-sensitivity (Fig. 3B). An increase in cell size of the mother cells in the BY4741 WT cells was observed, as in the W303 WT cells (data not shown). These results suggested that the ethanol-sensitivity of the mutants and the importance of the G2-M regulating system are dependent on genetic background. To investigate the ethanol-sensitive mutants in the BY4741 strain, we used a concentration of 6–11%. We identified 256 genes that are important for cell growth in the presence of 11% ethanol. Furthermore, 181 genes (underlined in Table 1) in the selected 256 genes were important for cell growth in the presence of 8% ethanol. The 256 genes selected were classified into twelve groups according to the presumed function of the gene product based on information from the SGD (*Saccharomyces* Genome Database) (Table 1). These results indicated that various biological functions are important for cell growth in the presence of ethanol.

What is the primary signal for the cell cycle delay? The addition of ethanol caused a delay in the cell cycle associated with transient dispersion of F-actin, resulting in an increase in cell size. It is possible that ethanol activates the morphogenesis checkpoint[7,8] monitoring the establishment of polarity in bud formation in budding yeast. The perturbation of actin localization by ethanol might be a primary signal for induction of the cell cycle delay. It would be interesting to investigate the relationship between the morphogensis checkpoint and the genes identified. Furthermore, since depolarization of F-actin has been observed on exposure to heat or osmotic stresses,[9,10] it is interesting to investigate whether the genes identified in this study are important for cell growth under such conditions.

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