Deregulation of DSE1 Gene Expression Results in Aberrant Budding within the Birth Scar and Cell Wall Integrity Pathway Activation in Saccharomyces cerevisiae

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Strains of Saccharomyces cerevisiae lacking Isw2, the catalytic subunit of the Isw2 chromatin remodeling complex, show the mating type-independent activation of the cell wall integrity (CWI) signaling pathway. Since the CWI pathway activation usually reflects cell wall defects, we searched for the cell wall-related genes changed in expression. The genes DSE1, CTS1, and CHS1 were upregulated as a result of the absence of Isw2, according to previously published gene expression profiles (I. Frydlova, M. Basler, P. Vasicova, I. Malcova, and J. Hasek, Curr. Genet. 52:87–95, 2007). Western blot analyses of double deletion mutants, however, did not indicate the contribution of the chitin metabolism-related genes CTS1 and CHS1 to the CWI pathway activation. Nevertheless, the deletion of the DSE1 gene encoding a daughter cell-specific protein with unknown function suppressed CWI pathway activation in isw2Δ cells. In addition, the deletion of DSE1 also abolished the budding-within-the-birth-scar phenotype of isw2Δ cells. The plasmid-driven overexpression proved that the deregulation of Dse1 synthesis was also responsible for CWI pathway activation and manifestation of the budding-within-the-birth-scar phenotype in wild-type cells. The overproduced Dse1-green fluorescent protein localized to both sides of the septum and persisted in unbudded cells. Although the exact cellular role of this daughter cell-specific protein has to be elucidated, our data point to the involvement of Dse1 in bud site selection in haploid cells.

The yeast cell wall is a crucial extracellular organelle that protects the cell from lysis during environmental stresses and morphogenesis. The cell wall of the budding yeast Saccharomyces cerevisiae is composed of glucans, mannoproteins, and a small amount of chitin (13, 14). To prevent lysis during cell expansion, the cell wall assembly and the cell cycle progression are coordinated by the mitogen-activated protein kinase (MAPK) Slt2 that is a component of the cell wall integrity (CWI) pathway. This pathway is thus periodically activated during budding at the time of polarized growth, but its activation also reflects cell wall damage (15, 35).

The critical point for cell integrity maintenance is the mother-daughter cell separation following cytokinesis when a controlled action of synthetic and degrading enzymes is necessary to prevent cell lysis. Chitinase Cts1 secreted by the daughter cell dissolves the primary chitin septum covered on both sides with a secondary septum and, in collaboration with other cell wall-degrading enzymes, allows the release of the daughter cell, leaving the mother cell with a prominent chitin bud scar and the daughter cell with a much less conspicuous birth scar (2). It has been reported that the birth scar contains little or no chitin (1, 23), although the exact composition of this structure is unknown. In haploid cells, the bud is formed next to the birth scar outwards, and the birth scar forms a zone restricted for budding (4, 29). Besides chitinase, other daughter cell-specific proteins were identified to participate in the cell separation.

Dse2 and Scw11 function as glucanases that help to degrade the cell wall from the daughter side of the septum (3, 5). Another protein, Dse1, was suggested to participate in pathways regulating cell wall metabolism, since some diploid strains bearing the dse1 deletion show a cell separation defect and display an increased sensitivity to drugs affecting the cell wall (6). The transcription of DSE1, as well as other daughter cell-specific genes, is Ace2 regulated and peaks in the early G1 phase asymmetrically, in the daughter cells only, due to the localization of the transcription factor Ace2 in the daughter cell nucleus (5). Furthermore, Dse1 interacts in the high-throughput two-hybrid analysis with the bud formation proteins Boi1 and Boi2 (8). However, the precise function of Dse1 is unknown.

Isw2, a member of the ISWI (initiation switch) class of ATPases, was first shown to be a component of a two-subunit (together with Itc1) complex with nucleosome-stimulated ATPase and ATP-dependent nucleosome spacing activities (32). The Isw2 chromatin remodeling complex is involved in the transcriptional regulation of a broad spectrum of genes by influencing the accessibility of chromatin to the transcriptional machinery (32). It was found to participate, e.g., in the repression of the mitotic cyclin-encoding gene CLB2 (24) and MATA-specific genes in MATA cells (20, 31). The absence of either Isw2 or Itc1 in MATA cells results in an inappropriate a-factor production and an autocrine activation of the pheromone response pathway. As a result, the pheromone-inducible genes are upregulated and cells display altered morphology resembling budding “shmoos” (20, 28, 30, 31). However, the level of derepression of MATA-specific genes in isw2Δ MATA cells as a direct effect of the Isw2 absence is very low. Their derepression is potentiated by the activated pheromone re-
sponse pathway. We proved it by microarray analyses comparing the expression profile of the isw2Δ MATα strain (versus the wild-type strain) with the expression profile of the isw2Δ ste4Δ MATα strain (versus the ste4Δ MATα strain), in which the pheromone response pathway is disrupted (9). The absence of the Isw2 complex also results in the phosphorylation of the Slt2 kinase and the activation of the CWI pathway which does not depend on the mating type (20).

Here, we report on a connection between the daughter cell-specific protein Dse1 and an aberrant budding within the birth scar. We found that the deletion of the DSE1 gene suppressed CWI pathway activation and abolished the aberrant budding of isw2Δ cells. Consistently, the plasmid-driven overexpression of the DSE1 gene in wild-type cells induced the budding-within-the-birth-scar phenotype as well as CWI pathway activation. Our data suggest that a tight temporal and spatial control of the daughter cell-specific Dse1 protein distribution is essential for maintenance of the birth scar as a zone forbidden for budding.

**MATERIALS AND METHODS**

**Strains, plasmids, media, and general methods.** The *S. cerevisiae* strains used in this study are listed in Table 1 and were derived from either BY4741 and BY4742 (Euroscarf collection) or S288C (yeast green fluorescent protein [GFP] clone collection; Invitrogen). The isw2Δ HIS3MX strain was prepared by replacing the ISW2 gene in BY4742 with the HIS3MX cassette as described previously (31). Deletion strains expressing specific GFP fusions from the chromosomal locus and double deletion strains were generated by mating, subsequent sporulation on Fowells medium, and spore dissection using the Singer micromanipulator. Enschachia coli DH5a [F- recA1 supE44 endA1 hsdR17 (rK- mK-) gyrA96 relA1 thi-1 (lacZYA-argF)U169deor R (80lacZAM15)] was used as the host in cloning procedures.

All the plasmids used in this study carried the galactose-inducible promoter GAL1. The empty vector pYC2/CT was purchased from Invitrogen, and pGAL-DSE1-GFP was constructed and kindly provided by David E. Stone (University of Illinois, Chicago, IL). The plasmid pGAL-DSE1-GFP was constructed as follows. The DSE1 coding region was amplified from the S288C chromosomal DNA using primers Dse1Fwd (5′-CGCCCGGATCCATGCGACGACATAAACTACT) and Dse1Rev (5′-GGCGGGATCCGACGACACAAGTATTGCAAGTAAAAGTAAAG) and Phusion DNA polymerase (Finnzymes, Finland). The purified PCR product was digested sequentially with SacI and SalI and ligated into the SacI/SalI-cut vector pYC2/CT-GFP that we constructed earlier (our unpublished data). The resulting construct was transferred into the BY4741 strain and checked for the production of the Dse1-GFP fusion protein by Western blotting and fluorescence microscopy.

Standard bacterial cultivation media and temperatures were used (21). Yeast cultures were grown at 30°C in eitherYPD (1% yeast extract, 2% peptone, 2% glucose), SC (0.17% YNB without amino acids and ammonium sulfate, 0.5% ammonium sulphate, 2% glucose, supplemented with a complete mixture of amino acids), or SC lacking uracil to maintain the selection for plasmids. The corresponding solid medium contained 2% agar. For the overexpression analyses, cells were pregrown overnight in SCraff-Ura (SC with 2% raffinose as the carbon source and lacking uracil) and then shifted for 4 to 6 h to SCGal-Ura (SC with 1% raffinose and 2% galactose and lacking uracil).

Standard methods were used for all DNA manipulations (21), bacterial cells were transformed by electroporation according to the protocol of Dower et al. (7), and yeast transformation was carried out by the lithium acetate method (10).

**Cell wall staining.** Cell wall staining was performed according to the protocols described in references 11 and 23. For double labeling of the cell wall structures, yeast cells were washed with potassium phosphate-citric acid (KCP) buffer (pH 5.9). To 20 μl of the cell suspension in KCP buffer (10^7 cells/ml), an aliquot of calcifluor white (American Cyanamid Co.) stock solution was added (final concentration, 0.5 μg/ml). Further, an aliquot of 5 μl of fluorescein isothiocyanate (FITC)-labeled wheat germ agglutinin (WGA-FITC) stock solution (Sigma) was added (final concentration, 100 μg/ml). The cells were then washed two times with KCP buffer and subjected to microscopic analysis.

**Western blot analyses.** Lysates were prepared according to a modified protocol of Riezman et al. (19). Yeast cells (optical density at 600 nm, 5 to 6) were collected by centrifugation, resuspended in fresh medium with phosphate and kinase inhibitors (100 mM NaF, 100 mM Na3VO4, 4 mM sodium orthovanadate, 100 mM β-glycerophosphate), and frozen in liquid nitrogen. Lysis was done by adding an equal volume of 3.7 M NaOH for 10 min on ice. The proteins were precipitated by incubation with trichloroacetic acid for 10 min on ice and collected by centrifugation at full speed in a microcentrifuge. The protein pellets were neutralized by 1 M Tris base and dissolved in sodium dodecyl sulfate sample buffer. After being separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, the proteins were transferred to nitrocellulose membranes. The detection of phosphorylated Slt2 was performed with anti-phospho-p44/42 MAPK (Thr202/Tyr204) antibody (Cell Signaling Technology). The blots were stripped and reprobed with specific anti-Mpk1 (y-244) antibody (Santa Cruz Biotechnology). The pixel densities of the bands were quantified using Adobe Photoshop image software, and the level of Slt2 phosphorylation was normalized for loading using the level of total Slt2p. The statistics represent the results from two independent experiments and at least two different loading amounts for each experiment. The expression of the Dse1-Myc and Dse1-GFP fusion proteins was followed using the anti-Myc tag (Cell Signaling Technology) and anti-GFP (Santa Cruz) antibodies, respectively.

**Microscopy.** The cells were inspected after washing with SC medium or KCP buffer, mounting on coverslips, and coating with a slice of 1% agarose in SC medium. The distribution of GFP fusion proteins or fluorescent probes was analyzed with a 100× PlanApochromat objective (numerical aperture, 1.4) using
RESULTS

Deletion of the DSE1 gene overrode CWI pathway activation in \( isw2^{\Delta} \) cells. Among genes whose expression was characterized at least twofold in the absence of \( isw2^{\Delta} \), there were three cell wall-related genes, \( CTS1, CHS1, \) and \( DSE1 \), and all were upregulated (9). We hypothesized that changes in the expression of these genes might account for an activation of the CWI pathway reported for \( isw2^{\Delta} \) cells previously (20).

Here we tested by Western blotting whether the CWI pathway was also activated in the \( isw2^{\Delta} stc4^{\Delta} MATa \) strain, lacking indirect defects induced by an autocrine activation of the pheromone response pathway (31). As shown in Fig. 1A, Slt2 phosphorylation reflecting CWI pathway activation was increased not only in the \( isw2^{\Delta} \) cells of both mating types but also in the \( isw2^{\Delta} stc4^{\Delta} MATa \) double mutant. To test our notion that the excess of the \( CHS1, CTS1, \) and \( DSE1 \) gene products might account for CWI pathway activation in the \( isw2^{\Delta} \) mutants, we prepared double deletion mutants lacking the \( ISW2 \) gene and one of the three selected genes and analyzed them for the level of phospho-Slt2. As shown in Fig. 1B, the additional absence of \( CHS1 \) or \( CTS1 \) genes did not significantly decrease the Slt2 phosphorylation observed in the single \( isw2^{\Delta} \) deletant. Quantification of the immunoblots proved that the level of Slt2 phosphorylation in the \( isw2^{\Delta} chs1^{\Delta} \) deletion mutant was about 115% (±6%) of that of the single \( isw2^{\Delta} \) deletion mutant and about 99% (±15%) for the \( isw2^{\Delta} cts1^{\Delta} \) mutant in comparison with the \( isw2^{\Delta} \) mutant level. These data showed that the deregulation of the chitin metabolism-related genes \( CTS1 \) and \( CHS1 \) was not responsible for CWI pathway activation in \( isw2^{\Delta} \) cells. In the last tested \( isw2^{\Delta} stc4^{\Delta} \) double deletion mutant, the Slt2 kinase was much less phosphorylated (67.7 ± 4%) in comparison with the single \( isw2^{\Delta} \) mutant (Fig. 1B). This result indicated that the upregulation of the \( DSE1 \) gene contributed to the cell wall integrity-signaling phenotype of \( isw2^{\Delta} \) cells.

\( isw2^{\Delta} \) cells displayed the budding-within-the-birth-scar phenotype dependent on \( DSE1 \). To analyze the cell wall structure of \( isw2^{\Delta} \) strains by fluorescence microscopy, we double labeled the cells with calcofluor white and WGA-FITC. Both dyes are routinely used for visualization of the yeast cell wall chitin; however, calcofluor white obviously does not label the residual chitin of the birth scar (17). The \( isw2^{\Delta} MATa \) cells displayed an aberrant morphology and a complex distribution of the cell wall chitin involving its accumulation at the base of the shmoofike projections (Fig. 2) as a consequence of an autocrine activation of the pheromone response pathway (31). The pattern of the cell wall labeling of the \( isw2^{\Delta} MATa \) cells was similar to that of the wild-type cells. No abnormal deposition of chitin was observed, and chitin was detected only in bud and birth scars. However, we came across an intriguing feature of birth scars in these cells. The birth scars that normally expand and fade with age (17) expanded but did not fade in the mutant cells. In the population of \( isw2^{\Delta} MATa \) cells, we found old cells with enlarged birth scars which were not observed in the wild-type cells. In addition, we discovered an interesting phenomenon that 49.5% of the \( isw2^{\Delta} MATa \) mother cells in the population formed buds inside the area bordered by the birth scar. No such cells were found in the wild-type population. Observations of the aberrant budding in the \( isw2^{\Delta} stc4^{\Delta} MATa \) cells proved that this phenotype was mating type independent (Fig. 2).

The birth scar normally forms a zone restricted for budding (29). In this respect, we considered that aberrant budding within the birth scar might be the reason for CWI pathway activation. In such a case, deletion of the \( DSE1 \) gene in \( isw2^{\Delta} \) cells that suppressed Slt2 phosphorylation should also prevent the budding phenotype of this mutant. Therefore, we doubled labeled the \( isw2^{\Delta} stc4^{\Delta} \) cells with calcofluor white and WGA-FITC. Indeed, the budding pattern of these cells was similar to wild-type cells because they displayed neither extremely enlarged birth scars nor the budding-within-the-birth-scar phenotype (Fig. 3). These data indicated that deletion of the \( DSE1 \) gene in the \( isw2^{\Delta} \) genetic background overrode the budding-within-the-birth-scar phenotype and suppressed activation of the CWI pathway.

The ectopic expression of \( DSE1 \) in wild-type cells induced the budding-within-the-birth-scar phenotype and CWI pathway activation. Supposing that the budding-within-the-birth-scar phenotype and the activation of the CWI pathway in \( isw2^{\Delta} \) cells were caused by an increased expression of the daughter cell-specific gene \( DSE1 \), its overexpression from a plasmid should induce the same phenotype. We transformed the wild-type cells with the plasmid pGAL-DSE1-Myc. As confirmed by
Western blotting, this strain produced Myc-tagged Dse1 at high levels under inducing conditions in the galactose medium (data not shown).

First, we checked the level of CWI pathway activation. We prepared lysates from cells carrying the plasmid pGAL-DSE1-Myc and control cells carrying the empty vector. Then, we analyzed the level of Slt2 phosphorylation by Western blotting. Indeed, our analysis proved that there was a significant increase in Slt2 phosphorylation in the cells with the induced expression of the DSE1 gene (Fig. 4A).

Further, we examined microscopically the wild-type cells carrying either the empty vector or the plasmid pGAL-DSE1-Myc cultivated in the galactose medium. They were double labeled with calcofluor white and WGA-FITC (Fig. 4B). Whereas the wild-type cells containing empty vector (WTa+pGAL) formed buds adjacent to the birth scars, the cells with over-expressing Dse1 (WTa+pGAL+DSE1) formed buds within the birth scars. This result suggests that Dse1 is involved in the regulation of CWI pathway.

FIG. 2. Wild-type cells formed buds next to the birth scar, whereas strains lacking Isw2p displayed bud scars within the birth scar. Cells were cultivated in liquid YPD medium overnight and double labeled with calcofluor white and WGA-FITC. The arrows indicate the birth scars in the wild-type (WTa; strain BY4741), isw2Δ MATa (isw2Δ; strain CRY3), and isw2Δ ste4Δ MATa (isw2Δste4Δ; strain CRY146) strains and chitin at the base of the shmoo-like projection in the isw2Δ MATa mutant (isw2Δ; strain CRY4). DIC, differential interference contrast. Bar, 5 μm.

FIG. 3. The deletion of the DSE1 gene in the isw2Δ mutant (isw2Δa) background abolished the budding-within-the-birth-scar phenotype. In contrast to the isw2Δ cells (strain CRY3), the isw2Δ dse1Δ mutant (isw2Δdse1Δa; strain CRY671) displayed a common axial budding pattern, i.e., the bud was formed just outside of the birth scar. Cells were cultivated in the liquid YPD medium overnight and double labeled with calcofluor white and WGA-FITC. The arrow indicates the birth scar. DIC, differential interference contrast. Bar, 5 μm.
produced Dse1 (WTa+/pGAL-DSE1) displayed enlarged birth scars. In addition, they showed also the budding-within-the-birth-scar phenotype. Moreover, after a serial passage in the galactose medium, Dse1 overproduction induced this phenotype in 97% of the mother cells in the population. We concluded that the overproduction of Dse1 was responsible for the activation of the CWI pathway as well as for the induction of the budding-within-the-birth-scar phenotype in the wild-type cells.

Dse1-GFP produced from the plasmid localized to both sides of the septum. DSE1 is referred to as a cell cycle-regulated gene that is expressed in the early G1 phase asymmetrically in daughter cells only (5). To analyze the distribution of Dse1-GFP in the wild-type cells (12), the cells were cultivated in YPD overnight, then transferred to fresh SC medium, and further cultivated for 3 h under vigorous shaking. There was no Dse1-GFP-specific fluorescence localized in the unbudded mother cells (Fig. 5A) and the cells with enlarging buds (Fig. 5B and C). The specific signal of the Dse1-GFP fluorescence was observed in separating cells as a ring-like structure associated with the daughter side of the septum (Fig. 5D) and in freshly separated daughters (Fig. 5E).

Since we found DSE1 upregulated in isw2Δ cells, we wanted to analyze Dse1 localization in these cells. We constructed the isw2Δ MATa strain producing the Dse1-GFP fusion from the chromosomal locus. In most of the isw2Δ MATa cells, Dse1-GFP was localized as a ring at the daughter side of the septum (as described above; Fig. 5). In addition, approximately 5% of the isw2Δ MATa cells with the localized Dse1-GFP signal displayed Dse1-GFP rings associated also with the mother side of the septum (Fig. 6B). This phenomenon was never found in the wild-type cells. Furthermore, Dse1-GFP frequently persisted at the separation sites (scars) of daughter (birth scar) as well as mother (bud scar) cells in isw2Δ MATa cells (Fig. 6D). Our results thus indicated that in isw2Δ MATa mutants, daughter cell-specific Dse1 protein was expressed and localized at the septum not only in daughters but also in mother cells.

Further, we wanted to analyze the distribution of Dse1 protein produced from the plasmid. Microscopic analyses of the cells carrying the plasmid pGAL-DSE1-Myc revealed that Dse1-Myc localized to both sides of the septum (data not shown). To analyze the distribution of overproduced Dse1 in living cells, we constructed the vector pGAL-DSE1-GFP. Whereas the separating wild-type cells showed Dse1-GFP localized only to the daughter side of the septum (see Fig. 5), the
separating cells overproducing Dse1-GFP displayed the fusion protein as a double ring at the septum (Fig. 7A). Dse1-GFP persisted at the cell surface after separation (Fig. 7B) and even after the initiation of new budding (Fig. 7C to E). Our data thus showed that the deregulated expression of DSE1 resulted in the persistence of Dse1 in cells after separation which could not be observed in wild-type cells and that the signal for Dse1 localization was present in the mother cells.

**DISCUSSION**

The local remodeling of the cell wall during the separation of *S. cerevisiae* cells ensured by the balance of cell wall synthetic and degrading activities depends, e.g., on the expression of several genes controlled by the transcription factor Ace2, a member of the CLB2 cluster (26). Ace2 is expressed in the G₂ phase of the cell cycle, and then, it is specifically localized by the Cbk1-Mob2 complex to the daughter nucleus only, where it induces the expression of daughter cell-specific genes (5, 36). Their transcript levels peak in the early G₁ phase (26). Here we bring evidence that the temporal and spatial deregulation of daughter cell-specific DSE1 gene expression results in CWI pathway activation and the budding-within-the-birth-scar phenotype.

We identified the budding-within-the-birth-scar phenotype of the DSE1 gene deregulation due to our detailed study of the isw2Δ cells showing an upregulation or a derepression of the
Dse1-GFP Calcofluor merge DIC

A

B

C

D

FIG. 6. In separating isw2Δ MATa cells (strain CRY557), Dse1-GFP was observed either as a ring at the daughter side of the septum (A) or forming rings on both sides of the septum (B). Dse1-GFP persisted at the cortical domain of daughter and mother cells after their separation (C). In addition, Dse1-GFP persisted at the cortical domain of a mother cell already showing a bud scar. (D) The isw2Δ MATa cells expressing GFP-tagged Dse1 from the chromosomal site were cultivated in YPD overnight, then transferred to SC medium, and further cultivated under vigorous shaking for 3 h. Cells were stained with calcofluor white and analyzed for Dse1-GFP and calcofluor white fluorescence. The arrows indicate the localization of Dse1. DIC, differential interference contrast. Bar, 5 μm.

DSE1 gene (9). Isw2 is a component of a two-subunit (together with Itc1) complex with nucleosome-stimulated ATPase and ATP-dependent nucleosome-spacing activities (32). Sherriff et al. (24) reported that Isw2 cooperates with the Fkh2 and Fkh1 transcription factors to repress the transcription of the B-type cyclin gene CLB2. Cdc28 bound to Clb2 phosphorylates Ace2, the main transcriptional activator of DSE1 gene expression, preventing its transport to the daughter cell nucleus (22).

FIG. 7. Overproduced Dse1-GFP localized to both sides of the septum forming a double ring in dividing cells (A), the signal persisted at the same sites during cell separation (B), and it was detected there even after the initiation of new budding (C to E). Cells carrying the pGAL-DSE1-GFP plasmid (strain CRY979) were cultivated overnight in SCraff-Ura and then shifted to SCGal-Ura for 4 h. DIC, differential interference contrast. Bar, 5 μm.
and thus a subsequent \textit{DSE1} transcription. If the loss of Isw2 leads to a derepression of \textit{CLB2}, it should result in an inhibition of \textit{DSE1} expression rather than in its activation that was monitored by our DNA microarray analysis (9). A higher level of \textit{DSE1} mRNA in \textit{isw2Δ} cells cannot be a result of its activation by Ace2. Rather, there could be another way for Isw2 to control \textit{DSE1} gene expression. Doolin et al. (6) reported that the disruption of \textit{ACE2} almost completely abolished the expression of \textit{DSE1}, but disrupting \textit{SWI5} led to an increase in \textit{DSE1} expression of at least 25\% over wild-type levels in the presence of an intact \textit{ACE2} gene. Such a remarkable inhibitory effect of this transcriptional activator suggests that Isw2 might have a direct role as a repressor in the regulation of the \textit{DSE1} gene or that it might be a mediator of the action of another yet-unknown repressor. In the \textit{DSE1} 5′ untranslated region, there are several binding sites not only for Ace2 but also for Fkh1 and Fkh2 transcriptional regulators. It is tempting to speculate that Isw2 can bind to the \textit{DSE1} 5′ untranslated region, either directly or throughout either Fkh protein (like in the \textit{CLB2} regulatory region) or Swi5, and thus influence chromatin structure in this region and, as a consequence, also \textit{DSE1} expression. In the absence of Isw2, this region would be remodeled and \textit{DSE1} transcription derepressed (like in the absence of Swi5), however, not completely induced like under the action of the activator Ace2.

The budding-within-the-birth scar is an unusual phenotype, since the area inside the birth scar is believed to be an exclusion zone for budding. In wild-type cells, the first bud scar can very rarely (∼1\%) overlap the birth scar; however, the bud scar has never been observed completely within the birth scar (4, 29). Under a deregulation of \textit{DSE1}, the area of the birth scar loses restriction, and there is a feasibility to form buds in this zone of inhibition. Data on the budding-within-the-birth-scar phenotype are very rare in the literature. In this respect, during the search for genes affecting bipolar budding, deletion mutants with mutations in two genes involved in splicing, \textit{IST3} and \textit{BUD13}, were reported to display individual bud sites adjacent to, overlapping, or within the birth scar (16). However, the mechanism behind this defect is unknown. Recently, the budding-within-the-old-division-site phenotype was found in cells lacking Rga1p (29). Rga1p is a GTPase-activating protein for Cdc42 that specifically prevents Cdc42 activation at the previous division site, thus establishing an exclusion zone that blocks subsequent polarization within that site. In \textit{rga1Δ} cells, Cdc42 remains in its GTP-bound state at the same place and new buds are nearly always formed at the old division site, thus displaying concentric bud scars at one pole of the cell. In contrast, cells with deregulated Dse1 (\textit{isw2Δ} cells and cells overexpressing \textit{DSE1}) displayed one or several bud scars within the birth scar region, and they had a normal localization of Rga1p-GFP (our unpublished data). This suggests that the Dse1-related budding-within-the-birth-scar phenotype is not a result of the absence of Rga1. More likely, it might be a consequence of problems in cell polarity signaling, since the protein Dse1 that we identified as being responsible for this phenotype was found to interact in high-throughput two-hybrid assays (not verified) with the Boi1, Boi2, and Zds2 proteins implicated in polar growth (8). Purevdorj-Gage et al. (18) showed that \textit{S. cerevisiae} cells under the influence of low-shear microgravity exhibit problems in polarity establishment, ranging budding, and increased aggregation accompanied with the increased expression of \textit{BUD5} and the decreased expression of daughter cell-specific genes \textit{DSE1}, \textit{DSE2}, and \textit{EGT2}. A connection of Dse1 with the cell polarity machinery might also be behind the detected activation of the CWI pathway in \textit{isw2Δ} cells, since the Dse1-interacting protein Zds2 was found to interact in high-throughput two-hybrid assays (8, 33) with Pkc1, a central component of the CWI pathway. Another indication of links between Dse1 and protein kinase C comes from the recent work of Sprowl et al. (27). They found that expression of bovine protein kinase Cα in \textit{S. cerevisiae} resulted in the growth inhibition of \textit{G2/M} cells with defects in septum formation and decreased the expression of daughter cell-specific genes \textit{CTS1}, \textit{DSE1}, and \textit{DSE2}. The ability of Dse1 to suppress an osmosensitive phenotype of the \textit{cdc37-34} mutant (34), most probably by adaptive changes of the cell wall, further suggests that Dse1 might have a signaling role in cell wall metabolism. Hypothetical WD40 repeats enhancing protein-protein interactions were identified by computer analysis in the Dse1 molecule and also support the idea that Dse1 might have a function in signal transduction.

Here, we demonstrate an interesting effect of the deregulation of the \textit{DSE1} gene leading to the budding-within-the-birth-scar phenotype and the activation of the CWI pathway. The elucidation of Dse1 links to potential interacting proteins and to other proteins localized to similar areas of daughter cells, e.g., Cwp1 protein that is specifically targeted to the birth scar area (25), is an important task to be undertaken in the near future. It may help to uncover the precise function of the Dse1 protein and its role in cell morphogenesis.

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