Emw1p/YNL313cp is essential for maintenance of the cell wall in *Saccharomyces cerevisiae*

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There are six essential genes in the *Saccharomyces cerevisiae* genome which encode proteins bearing the tetratricopeptide repeat (TPR) domain that mediates protein–protein interaction. Thus far, the function of one of them, *YNL313c*, remains unknown. Our conditional mutants of *YNL313c* display osmoremedial temperature sensitivity, hypersensitivity to both Calcofluor White and low concentrations of SDS, and osmoremedial caffeine sensitivity. These are hallmarks of mutants that display cell wall defects. Accordingly we rename the gene as *EMW1* (essential for maintenance of the cell wall). Loss of Emw1p function is not associated with abrogation of the cell wall integrity (CWI) MAP kinase cascade. Instead, *emw1ts* mutants activate this cascade even at permissive temperature, indicating that loss of Emw1p function does not cause a defect in sensors and effectors of cell wall signalling, but leads to a cell wall defect directly. Constitutive activation of the CWI cascade is reflected by the overproduction of chitin by *emw1ts* mutants, a compensatory response frequently displayed by cell wall mutants. Growth is restored to *emw1ts* mutants incubated at otherwise non-permissive temperature when *GFA1* is overexpressed. *GFA1* encodes the hexosephosphate aminotransferase that catalyses the rate-limiting step in the pathway that synthesizes the chitin precursor UDP-GlcNac. The possibility that Emw1p is required for function of Gfa1p was ruled out, because the *emw1ts* phenotype persists when the requirement for Gfa1p is bypassed. Furthermore, if loss of Emw1p function leads to loss of function of Gfa1p, then chitin synthesis would be diminished. Instead, a stimulation of the synthesis of this polymer is detected. Consequently, the defect associated with *emw1ts* mutants may be associated with compromise in one of the remaining processes that depend on UDP-GlcNAc, namely N-glycosylation or glycosylphosphatidylinositol (GPI)-anchor synthesis.

**INTRODUCTION**

The tetratricopeptide repeat (TPR) is a structural motif originally identified in yeast as a protein–protein interaction domain (Sikorski et al., 1990). This motif consists of three to 16 tandem repeats of 34 amino acids. Though there is a great deal of degeneracy in the motif, structural analysis indicates a highly conserved 3D structure (D’Andrea & Regan, 2003). The huge number of protein–protein interactions in yeast revealed by proteome-wide two-hybrid and tandem affinity purification (TAP)-tag datasets indicate the extent to which cell physiology relies on the formation of multi-protein complexes, and it has been estimated that there are five interactions per protein on average (Grigoriev, 2003).

Twelve-three proteins in *Saccharomyces cerevisiae* bear TPR domains, of which six are essential (D’Andrea & Regan, 2003). Three of these six essential proteins are Cdc27, Cdc23 and Cdc16, components of the anaphase-promoting complex (APC/cyclosome), the E3 ubiquitin-protein ligase required for both the metaphase to anaphase transition and subsequent exit from mitosis (Peters, 2006); Tfc4 is a subunit of the RNA polymerase III transcription initiation factor complex (TFIIC) (Geiduschek & Kassavetis, 2001), and Prp6 is a component of the U4/U6-U5 snRNP complex required for pre-mRNA splicing (Legrain et al., 1991). This leaves one essential TPR protein, encoded by *YNL313c*, for which there is no indication regarding its cellular role. Use of TPRpred (a tool for prediction of TPR repeats from protein sequences) indicates the presence of six TPR domains clustered at the C-terminal end of the protein (Karpelahalli et al., 2007). The remainder of the primary amino acid sequence does not bear recognized motifs from which a function for this 102 kDa protein could be inferred. Interrogation of the protein–protein interaction datasets available for *S. cerevisiae* reveals 21 binding partners involved in numerous
physiological processes; some of these interactions are bound to be spurious because their subcellular location does not match that of YNL313cp. For instance, there are putative interacting partners located in the mitochondria and the endoplasmic reticulum (ER), yet high-throughput localization of GFP fusions indicates that YNL313cp is found in the nucleus and cytoplasm (Hazbun et al., 2003; Huh et al., 2003).

As we could not infer a possible cellular role for YNL313c via rudimentary bioinformatics analysis or interrogation of the genome/proteome-wide datasets, we generated conditional mutants of the corresponding gene and assessed the phenotypes displayed by these mutants under non-permissive conditions. Compromising YNL313cp function led to phenotypes that are hallmarks of defects associated with synthesizing or maintaining the cell wall, and we rename the protein Emw1p (essential for maintenance of the cell wall) accordingly. We go on to show that the defect associated with loss of YNL313cp/Emw1p function is not associated with abrogation of the cell wall integrity (CWI) cascade, but is associated with a process that depends on the donor nucleotide sugar UDP-GlcNAc, an essential precursor for three processes, all of which are associated with cell wall homeostasis.

**METHODS**

**Strains, plasmids and media.** Yeast strains used in this study are listed in Table 1. Plasmids are described in the appropriate sections below. Strains were grown in either rich medium (YPD) or synthetic minimal medium (SD), with appropriate supplements for plasmid maintenance (Rose et al., 1990). Deletion of SLT2/MPK1 was performed by replacing this ORF with hph (conferring resistance to hygromycin B) via one-step gene replacement, as described by Goldstein & McCusker (1999). Integration at the correct locus was confirmed by PCR using appropriate primers.

**Isolation of EMW1 conditional alleles.** The heterozygous EMW1/ emw1Δ diploid Y21131 was obtained from EUROSCARF (Table 1) and transformed with a centromeric URA3 S. cerevisiae/Escherichia coli shuttle vector (pY33T-E) bearing the EMW1 promoter (a fragment 0.45 kb upstream of the ATG) fused to the EMW1 ORF (a 2.5 kb fragment), followed by the ADH1 transcriptional terminator (a 0.4 kb fragment). The EMW1 promoter and ORF from this vector were then subcloned into a centromeric LEU2 S. cerevisiae/E. coli shuttle vector (again bearing the ADH1 terminator), to give pY111T-E. Strain Y21131 was transformed with pY33T-E (to give SPD, Table 1).

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**Table 1. Strains used in this study**

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<tr>
<th>Strain</th>
<th>Description</th>
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<tr>
<td>SPD</td>
<td>MATα his3Δ1/ his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 MET15/ met15Δ0 ura3Δ0/ura3Δ0 emw1::kanMX4/EMW1</td>
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<td>SPE</td>
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</tr>
<tr>
<td>SP0</td>
<td>MATα his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0 MET15 emw1::kanMX4 bearing pY111T-E plasmid (EMW1/LEU2)</td>
<td>This study</td>
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<tr>
<td>SP1</td>
<td>SP0 bearing pY111T-e-1&lt;sup&gt;st&lt;/sup&gt; plasmid (emw1-1&lt;sup&gt;st&lt;/sup&gt;/LEU2) instead of pY111T-E</td>
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<td>SP0 bearing pY111T-e-2&lt;sup&gt;nd&lt;/sup&gt; plasmid (emw1-2&lt;sup&gt;nd&lt;/sup&gt;/LEU2) instead of pY111T-E</td>
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<td>SP0 bearing pY111T-e-3&lt;sup&gt;rd&lt;/sup&gt; plasmid (emw1-3&lt;sup&gt;rd&lt;/sup&gt;/LEU2) instead of pY111T-E</td>
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<td>SP0 bearing pY111T-e-4&lt;sup&gt;th&lt;/sup&gt; plasmid (emw1-4&lt;sup&gt;th&lt;/sup&gt;/LEU2) instead of pY111T-E</td>
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<td>SP0 bearing pY111T-e-5&lt;sup&gt;th&lt;/sup&gt; plasmid (emw1-5&lt;sup&gt;th&lt;/sup&gt;/LEU2) instead of pY111T-E</td>
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<td>SPG2</td>
<td>SP0 bearing pE-EMW1-GFP instead of pY111T-E</td>
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*EUROpean Saccharomyces Cerevisiae ARchive for Functional analysis.*
1). This strain was sporulated, followed by subsequent dissection of tetrads and selection of an emw1A haploid (strain SPE), with viability maintained by the presence of the URA3 vector bearing EMW1 (pY33T-E). Mutations in EMW1 were generated by error-prone amplification of the EMW1 ORF using pY33T-E as template, as described by Fenton et al. (2002). The oligonucleotide primers were designed so that the 5’ primer overlapped the 3’ end of the EMW1 promoter and the 3’ primer overlapped the 5’ end of the ADH1 transcriptional terminator (15 bp overlaps in both cases). Strains bearing the mutant emw1 alleles were generated by transforming strain SPE with (i) the mutant emw1 PCR products plus (ii) a large linear fragment from pY111T-E lacking the wild-type EMW1. The two fragments recombined with each other in vivo, owing to the 15 bp overlaps between them. The transformants, selected on SD medium lacking both leucine and uracil, possessed the pY33T-E URA3 vector bearing wild-type EMW1, and a LEU2 vector bearing mutated emw1. More than 4000 transformants were picked and incubated overnight at 25 °C in 384-well plates containing SD lacking leucine. The transformants bearing emw1” mutants were isolated as described previously (Hu et al., 2005). To ensure that these ts phenotypes were attributed to mutated EMW1, plasmids from the strains were recovered, transformed into the SPE strain and rescreened for the ts phenotype. In this way, a series of isogenic emw1A haploid strains were generated, possessing vector-borne copies of wild-type EMW1 (SP0, Table 1) or emw1” alleles (SP1–5, Table 1). The coding regions of the emw1” mutants were sequenced.

Fusions encoding Emw1p–GFP were assembled in pUG23 (kindly provided by J. Hegemann, Heinrich-Heine-Universität, Düsseldorf, Germany), a centromeric HIS3 vector that bears the MET25 promoter, to give pM-EMW1-GFP. The MET25 promoter in pM-EMW1-GFP was removed and replaced with the native EMW1 promoter to give pE-EMW1-GFP. The fusions maintained cell viability in the emw1A background, as assessed by growth on SD medium without histidine, but containing uracil (50 mg l⁻¹), giving strains SPG1 and SPG2 (Table 1).

Sensitivity to cell wall-damaging agents. Exponentially growing cultures in YPD were adjusted to equal cell density (1 × 10⁷ cells ml⁻¹) and four successive sixfold serial dilutions were spotted on YPD, or YPD containing the desired concentration of sorbitol, caffeine, Calcofluor White (CFW) or SDS. The plates were incubated at the temperatures specified in the figure legends.

Assessing multicopy suppression by selected ORFs. Candidate genes were overexpressed from the vector pY195M, which is a modification of the episomal URA3 vector YEpplac195 (Gietz & Sugino, 1988). The vector bears the MET25 inducible promoter and the transcription terminator from PKGI, EMW1, CHS3 and GFA1 were amplified from S. cerevisiae genomic DNA and ligated into pY195M, to give vectors pY195M-E, pY195M-C3 and pY195M-GF, respectively. The resulting vectors were transformed into the SP0 wild-type strain and the isogenic emw1-1” strain SP1 (Table 1). Expression of the gene was induced by incubation on SD plates lacking methionine.

Assessing the activation state of the MAP kinase (MAPK) CWI pathway. Exponentially growing cultures were either heat-shocked or treated with 10 mM caffeine (as specified in the figure legends). Preparation of total yeast protein extracts was described previously (Panaretou et al., 1998). Western blots were probed with either (i) anti-(Thr202/Tyr204)-p44/42 MAPK antiserum (New England Biolabs), which specifically recognizes the dually Thr190/Tyr192-phosphorylated (activated) Slt2p/Mpk1p in yeast, or (ii) anti-Mpk1/Slt2 (Santa Cruz). Activity of the Rlm1p transcription factor was assessed using p2-RLM1, a YIL117c promoter–LacZ reporter plasmid (Jung et al., 2002).

CFW staining and microscopy. Cells were grown at 25 °C in YPD to exponential phase and then incubated for 3 h at the temperatures indicated in the figure legends. Cells were then fixed with formaldehyde and stained with CFW as described by Phelan et al. (2006). Cells were viewed using a Leica DM RXA2 microscope equipped with differential interference contrast (DIC) optics and using an A4 narrowband 4’6-diamidino-2-phenyindole (DAPI) blue filter (340–380 nm excitation wavelength). Images were collected by an ORCA digital camera (Hamamatsu). Emw1p–GFP fusions were localized using a FITC filter.

RESULTS

Abrogating the function of YNL313cp leads to a catastrophic loss in CWI

To determine the function of YNL313c/EMW1, we generated conditional lethal alleles by PCR-mediated mutagenesis. Five emw1ts mutants were isolated, all exhibiting a robust ts phenotype (Fig. 1a). Three mutants were selected for further analysis, based on identifying the mutant with the slowest growth rate, the mutant with the fastest growth rate and a mutant displaying an intermediate growth rate, at permissive temperature. Therefore, the mutants selected were emw1-1”, emw1-5” and emw1-4”, which exhibited doubling times of 270, 150 and 180 min at 25 °C, respectively; this was in comparison with the 90 min doubling time displayed by the wild-type. The Emw1p coding regions were sequenced. Most error-prone PCR techniques are not truly random because they yield more transitions than transversions. The method we used, however, removes this bias (Fenton et al., 2002). The amino acid substitutions were: emw1-1”, L105S, V422G and Y435C; emw1-5”, L5S, F282L, K480E, L498P and R896G; and emw1-4”, S154P, I454T, Q511R, Q561L, K704E and S875T.

The emw1-1” cells (Fig. 1b) were rounder and larger than wild-type cells at both permissive (25 °C) and non-permissive (37 °C) temperatures. Increased cell size is typically associated with cell wall mutants (Levin, 2005). The growth defect of emw1ts cells at non-permissive temperature was alleviated by osmotically stabilizing the medium, and the mutants were sensitive to CFW and SDS, with emw1-1” exhibiting the most hypersensitive phenotype followed by emw1-4” and emw1-5” (Fig. 2a). The mutants also displayed osmomediated caffeine sensitivity (Fig. 2b). All of these phenotypes are hallmarks of cells with defects in maintaining integrity of the cell wall (Levin, 2005). CFW blocks chitin polymerization, resulting in a weakened cell wall (Ram & Klis, 2006). Caffeine blocks cell wall biosynthesis indirectly, and cell wall defects lead to sensitivity to low levels of SDS because a weakened cell wall allows the molecules of the detergent to penetrate more easily (Levin, 2005).

Loss of CWI in the emw1ts background is not due to a defect in the CWI pathway

The cell wall defect in emw1ts mutants may be due to compromised activity of proteins that sense and respond to

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cell wall damage. Of all the mechanisms that contribute to regulation of cell wall components, the most important is the CWI MAPK cascade. Cell surface sensors signal to the Rho1 GTPase, which activates the cascade, ultimately leading to cell wall remodelling. The pathway ends with phosphorylation of the Slt2p/Mpk1p MAPK, which subsequently phosphorylates and activates the transcription factor Rlm1 (Levin, 2005). This leads to increased transcription of genes encoding proteins that drive cell wall construction, such as CHS3 and FKS2, encoding chitin synthase 3 and a component of the β-1,3-glucan synthase, respectively (Jung & Levin, 1999). The MAPK Slt2p/Mpk1p kinase is

![Fig. 1. Phenotype of conditional emw1 mutants. (a) Strains lacking a genomic copy of EMW1, expressing a vector-borne copy of either the wild-type EMW1 or an emw1<sup>ts</sup> mutant, were streaked on YPD and incubated for 3 days at 25 or 37 °C. (b) EMW1 wild-type and the emw1-1<sup>ts</sup> mutant were grown in YPD at 25 °C to exponential phase. One aliquot of each culture was observed directly, another aliquot was shifted to 37 °C for 3 h prior to observation (cells observed at ×1000 magnification; bar, 25 μm).](http://mic.sgmjournals.org)

![Fig. 2. Loss of Emw1p function leads to defects in CWI. (a) EMW1 wild-type and emw1<sup>ts</sup> mutants were grown at 25 °C to exponential phase and diluted to equal cell density. Sixfold serial dilutions were spotted across YPD (in the direction of the arrow), or YPD containing sorbitol, CFW or SDS, followed by incubation at the temperatures indicated for 3 days. (b) The same cultures were spotted across YPD or YPD containing caffeine or caffeine plus sorbitol, followed by incubation at 30 °C for 3 days.](http://mic.sgmjournals.org)
phosphorylated and therefore activated in emwlts cells at non-permissive temperature (Fig. 3a). The same is true when cells are incubated in the presence of caffeine at permissive temperature (Fig. 3b). Moreover, Slt2p/Mpk1p is constitutively active in the emwlts background at permissive temperature in the absence of cell wall destabilizers, whereas we did not detect activated Slt2p/Mpk1p in wild-type cells (Fig. 3a, b). Not surprisingly, this is reflected by the activation state of the Rlm1 transcription factor under the same conditions (Fig. 3c, d). Accordingly, CWI signalling is not lost when Emw1p function is compromised. Constitutive activation of CWI signalling as the cause of the no-growth phenotype displayed by emwlts mutants at non-permissive temperature was ruled out, because the emwlts/slt2(Δ)Δ double mutant was inviable at 25 °C (Fig. 4). Constitutive activation of Slt2p/Mpk1p implies that emwlts mutants are experiencing cell wall stress. Cell wall mutants, such as those deficient in synthesis of β-1,3-glucan, β-1,6-glucan, mannosylation (cited in Bulik et al., 2003) and glycosylphosphatidylinositol (GPI) anchors (Sobering et al., 2004), exhibit an increased deposition of chitin in the lateral cell wall as part of a compensatory mechanism. As expected, an increase in chitin deposition was also displayed by emwlts cells (Fig. 5). Moreover, this increase in chitin deposition was observed at permissive as well as non-permissive temperatures (Fig. 5). In cell wall mutants, this is due to upregulation in activity of the chitin synthase Chs3p, which is responsible for making 90% of cellular chitin (Bulik et al., 2003). Not surprisingly, boosting this compensatory mechanism in the emwl1ts mutant, by ectopic overexpression of CHS3, suppressed the growth defect at non-permissive temperature (Fig. 6a).

**Gfa1 is a dosage suppressor of emwlts**

Thus far, our data indicate that Emw1p plays an essential role in cell wall homeostasis. This prompted a re-examination of the 21 binding partners of Emw1p identified from the protein–protein interaction datasets available for *S. cerevisiae*. In particular, we looked for binding partners that are found in the same cellular location as Emw1p. One of these binding partners is Gfa1p, a hexosephosphate aminotransferase that converts fructose 6-phosphate to glucosamine 6-phosphate, which is the rate-limiting step in the pathway that synthesizes UDP-GlcNAc. Proteome-wide localization studies indicate that Gfa1p (Kumar et al., 2002) and Emw1p (Hazbun et al., 2003; Huh et al., 2003) are both found in the cytoplasm. UDP-GlcNAc is the donor nucleotide sugar essential for

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Fig. 3. The MAPK CWI pathway is activated when Emw1p function is lost, and operates normally under conditions that lead to cell wall stress. *EMW1* and *emwlts* mutants were grown to exponential phase at 25 °C and then split into two aliquots, with one aliquot incubated for 1 h at 25 °C and the other aliquot subjected to heat shock (incubation for 1 h at 37 °C). The same cultures were grown to exponential phase at 30 °C and then incubated for 1 h in the presence or absence of 10 mM caffeine. Cells were harvested and levels of dually phosphorylated Slt2 (indicated as Slt2-P) and total Slt2 were detected on Western blots using appropriate antisera (a, b). The same strains were transformed with p2-RLM1, a vector bearing a *YIL117c* promoter–LacZ fusion. LacZ activity was assayed after cells were treated in the same way as described above. *YIL117c–LacZ* is a reporter for Rlm1-dependent transcription. Data on the y axis are presented as LacZ activity in Miller units; error bars, SEM (n=3).
three processes in yeast, all of which are essential for maintenance of the cell wall, namely N-glycosylation, GPI-anchor synthesis and chitin synthesis. The interaction of Gfa1p with Emw1p is physiologically significant, as overexpression of GFA1 rescued the null-growth phenotype of the \( emw1^{-1}\) mutant at non-permissive temperature (Fig. 6b). One interpretation of this result is that Emw1p is required for Gfa1 function. This could be tested because cells lacking Gfa1p are glucosamine auxotrophs. Exogenous glucosamine bypasses the need for Gfa1p, because the amine can enter cells and is subsequently phosphorylated by hexokinases. Indeed GFA1 was originally identified from a screen that selected for glucosamine auxotrophs (Watzele & Tanner, 1989; Whelan & Ballou, 1975). However, exogenous glucosamine did not suppress the null-growth phenotype of \( emw1^{-1}\), \( emw1^{-4}\) or \( emw1^{-5}\) at non-permissive temperature (not shown). Emw1p is also found in the nucleus, though we were doubtful of localization in this organelle, as examination of the images from the proteome-wide localization studies indicates very weak

Fig. 4. Synthetic lethality of \( emw1^{-1}/slt2\)\((mpk1)\). An \( emw1^{-1}\)\(\Delta\)\(slt2\(\Delta\)\(mpk1)\) strain bearing EMW1 on a URA3 vector was transformed with a LEU2 vector bearing wild-type EMW1 or the mutant \( emw1^{-1}\) alleles indicated in parentheses. Transformants were subsequently incubated on SD with 5-fluoroorotic acid for 3 days at 25 °C. An \( emw1\)\(\Delta\) strain with intact SLT2\(\Delta\)\(MPK1)\) bearing both EMW1\-URA3 and EMW1\-LEU2 vectors was also included (top-right sector).

Fig. 5. Excess and aberrant deposition of chitin when Emw1 function is compromised. Wild-type EMW1 and \( emw1^{-1}\) mutants were grown to exponential phase at 25 °C. Cultures were divided into two aliquots, one was maintained at 25 °C and the other was shifted to 37 °C for 3 h. Cells were fixed, prior to staining with CFW, and then observed under DIC optics and by fluorescence microscopy (F). Images were processed identically in order to preserve relative intensities of fluorescence. Bar, 25 μm.
nuclear fluorescence of Emw1p–GFP fusions. We examined the localization of our own Emw1p–GFP fusion, which retained the essential function of Emw1p as it was able to maintain the viability of emw1Δ cells. GFP fluorescence was weak overall, when expression of the fusion gene was driven by the EMW1 promoter, suggesting that native levels of Emw1p are low. Nevertheless, weak fluorescence in both the nucleus and the cytoplasm was detected (Fig. 7a). The nuclear localization was more evident, however, when expression of the fusion gene was driven by the inducible MET25 promoter (Fig. 7b).

**DISCUSSION**

The hitherto uncharacterized protein Emw1p does not bear motifs from which a function for this protein could be inferred. However, the protein does bear TPR domains, which are likely to mediate all or some of the interactions with the 21 other proteins identified from the protein–protein interaction screens applied to *S. cerevisiae*. Some of these interactions may be artefacts arising from the interaction screening methods. The biologically relevant binding partners may be those that share the most important property of Emw1p, namely that it is required for cell viability. Five of these interacting proteins are essential for viability. These are Rpt5 and Rpn11, which are components of the 26S proteasome (Rubin et al., 1998; Verma et al., 2002); Kar2, a chaperone resident in the ER (Nishikawa et al., 2001); Pta1, a component of the complex that carries out cleavage and polyadenylation of mRNA (Zhao et al., 1999); and Gfa1, which catalyses the rate-limiting step in the pathway that synthesizes UDP-GlcNAc, the donor nucleotide sugar for three processes known to be essential for CWI (Orlean, 1997).

We have provided compelling evidence that Emw1p plays an essential role in maintenance of the cell wall. However, this protein does not play a role in the CWI signalling pathway; indeed, loss of function leads to activation of this cascade. It is unlikely that Emw1p plays a role in the three other pathways that make a comparatively minor contribution to signalling that affects cell wall construction, for the following reasons. (i) Defects in the Rim101 pathway lead to sensitivity to SDS and caffeine (Castrejon et al., 2006); however, unlike EMW1, none of the genes encoding components of this pathway are essential (Penaña & Arst, 2002). Also, though rim mutants are sensitive to SDS and caffeine, they are resistant to CFW (de Groot et al., 2001). This is in contrast to emw1ts mutants, which display a CFW hypersensitive phenotype. (ii) The calcineurin pathway mounts a compensatory response when CWI is compromised. However,
abrogating this pathway by either deleting all genes encoding the *S. cerevisiae* homologues of calcineurin (Cyert et al., 1991), or deleting the gene encoding the calcineurin-responsive transcription factor Crz1p (Matheos et al., 1997; Stathopoulos & Cyert, 1997), is not lethal. Furthermore, deletion of the *Candida albicans* orthologue of CRZ1 renders cells sensitive to SDS, but has no effect on sensitivity to CFW (Santos & de Larrinoa, 2005). (iii) The high osmolarity (HOG) pathway also influences the plasticity of the cell wall. However, mutants in this pathway are resistant to CFW (Garcı´a-Rodriguez et al., 2000), in contrast to the hypersensitivity to CFW displayed by emw1ts mutants.

Activation of the CWI cascade in emw1ts mutants indicates that Emw1p plays a more direct role in cell wall biosynthesis. This is plausible, given that emw1ts mutants share a key phenotype with mutants that exhibit defects in the synthesis of the cell wall polymers β-1,3-glucan and β-1,6-glucan, namely an increase in chitin deposition (Bulik et al., 2003). It was not surprising, therefore, to find that GFA1 is a dosage suppressor of the emw1ts mutant, because Gfa1p catalyses the rate-limiting step in the pathway that synthesizes UDP-GlcNAc, the donor nucleotide sugar for three processes known to be essential for CWI, namely N-glycosylation, GPI-anchor synthesis and chitin synthesis. UDP-GlcNAc is the sole donor nucleotide sugar for chitin synthesis. Consequently, if the emw1ts mutation led to loss of Gfa1p activity, chitin synthesis would be diminished. Instead, we see a stimulation in synthesis of this polymer. Therefore, the defect in the emw1ts mutant is associated with a process that depends on UDP-GlcNAc, as opposed to a defect in its synthesis. This is credible because exogenous glucosamine fails to suppress the emw1ts growth defect at 37 °C. This leaves the production of GPI-anchored proteins and N-glycosylation as the remaining known uses of UDP-GlcNAc. Notably, the defects associated with compromised GPI-anchor biosynthesis are also suppressed by overexpression of GFA1 (Sobering et al., 2004). The only other recorded instance of dosage suppression by GFA1 is reversal of the phenotype caused by a defect in traffic of vesicles from the ER to the Golgi (Morsomme & Riezman, 2002); the cargo of these vesicles includes GPI-anchored proteins. The step-wise assembly of GPI anchors begins on the cytoplasmic face of the ER, with the transfer of GlcNAc from UDP-GlcNAc to phosphati-
dylinositol (PtdIns). Following deacylation of the GlcNAc moiety, the resulting glycolipid is flipped to the ER lumen, where the PtdIns is acylated, followed by addition of multiple mannose and phosphoethanolamine moieties, ending with transfer of the GPI anchor to a polypeptide C terminus (Orlean & Menon, 2007). Emw1p is located in the cytoplasm (Hazbun et al., 2003; Huh et al., 2003), so it could take part in the steps associated with GPI-anchor biosynthesis that take place on the cytoplasmic face of the ER membrane. That GPI anchoring is defective in emw1ts cells is further supported by closer examination of the mutant phenotype. Firstly, emw1ts cells display a cell separation defect, thereby forming aggregates (Figs 1 and 5).

This could be due to limited targeting of the GPI protein Egt2, which plays a role in degradation of the septum (Fujita et al., 2004). Secondly, emw1ts cells display increased deposition of chitin specifically in the lateral walls of mother cells (Fig. 5); the same is true in mutants lacking the GPI-anchored protein Ecm33, as well as in cells with defects in GPI anchoring itself (Pardo et al., 2004; Sobering et al., 2004). We also note that the GPI assembly pathway shares the UDP-GlcNAc activated sugar precursor with the dolichol pathway for N-glycosylation. N-Linked glycosylation begins on the cytoplasmic face of the ER, with the sequential addition of two GlcNAc moieties and five mannose moieties to the carrier dolichyl pyrophosphate. All of these steps are essential. Given its localization, Emw1p may be required for N-glycosylation steps on the cytoplasmic face of the ER membrane. It is conceivable that both N-glycosylation and GPI-anchor biosynthesis are defective in emw1ts cells. Ynl313cp/Emw1 may play a role in delivery of UDP-GlcNAc to the cytoplasmic face of the ER. Furthermore, defects in both N-glycosylation and GPI-anchor assembly lead to defects in the secretory pathway (Copic et al., 2009). Consequently, we would expect a genetic interaction between *EMW1* and components of the secretory pathway. In agreement with this is the recent finding that compromising the function of Sec15, which is required for the polarized targeting of vesicles to sites of endocytosis, is synthetically lethal in combination with limiting the expression of *EMW1* (Davierwala et al., 2005).

Emw1p is found in all fungi, and orthologues are also found across the eukaryotic lineage. Therefore, its essential function is likely to be associated with a core physiological process. As the protein is localized to the nucleus as well as the cytoplasm, it is possible that it interacts with different proteins in these two locations, these interactions being mediated by the six TPR domains found towards the C terminus of *Emw1p*. *S. cerevisiae* devotes a significant proportion of metabolic effort to synthesizing and maintaining the cell wall, with over 1000 genes displaying a cell wall phenotype when deleted (Klis et al., 2006). However, only a few of these genes are essential, owing to the redundancy associated with cell wall biosynthesis. For instance, there are three chitin synthases in *S. cerevisiae* (Lenardon et al., 2010). *EMW1* joins the select group of essential genes which are indispensable for maintaining the integrity of the cell wall.

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**REFERENCES**


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