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Old and new approaches used to identify gene products important for *Saccharomyces cerevisiae* cell wall biology

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The cell wall of *Saccharomyces cerevisiae* is required for cell viability under various environmental conditions. The functions of the wall are to prevent lysis under low osmolarity conditions, provide shape for the cell, and form a permeability barrier. The yeast cell wall is primarily composed of three classes of macromolecules: chitin, glucan, and mannoprotein. Chitin is a polymer of β-1,4-*N*-acetylglucosamine, glucan is a polymer of β-1,3-glucan with β-1,6-glucan branches, and the mannan component consists of highly mannosylated proteins. These three classes of molecules are covalently cross-linked to one another forming a matrix that is different from any structure found in mammalian cells. Because of these differences the cell wall is viewed as ideal territory to identify targets for antifungal drug discovery.

The cell wall composition/shape are quite dynamic — changing in response to heat, mating pheromones, hypotonic shock, and the cell cycle [2,6,11,16,23]. The cell wall integrity pathway is a communication conduit responsible for monitoring and controlling changes in the cell wall and mediating responses to the environment (Figure 1). This signaling pathway includes a mitogen-activated protein (MAP) kinase cascade consisting of Bck1, Mkk1, Mkk2, and Slt2 kinases. Strains lacking components of this MAP kinase cascade have a temperature-sensitive lytic phenotype consistent with the role of these proteins in the maintenance of cell wall integrity [1]. The MAP kinase pathway is regulated by kinase Pkc1, which is regulated by GTP binding protein Rho1. Rho1 and Pkc1 are each essential for cell viability [18,21,24]. The environmental sensors that activate the cell integrity pathway are Wsc1 and Mid2 [26,35]. These sensor proteins are membrane associated and contain Ser/Thr-rich extracellular domains that are predicted to form a rigid arm-like structure that extends out to the cell wall to sense disturbances [19,26]. The Mid2 extracellular domain is required for function [25]. Despite much study of this signaling pathway, the downstream effects are not well characterized. Rlm1 is a transcription factor activated by Slt2 and has only recently been shown to impact the transcription of 25 genes [15]. Redundant DNA-binding proteins, Nhp6A and Nhp6B, are important for maintaining cell wall integrity; however, their mechanism of action remains unknown [5]. Little else is known about the downstream impact of the cell wall integrity pathway.

The number of genes that are shown to have a role in cell wall structure, biosynthesis, or signaling is large. These gene products have been identified using several different approaches. A number of genes were identified *via* screens for mutant strains with altered sensitivity to calcofluor white [20,27]. Calcofluor white is a negatively charged fluorescent molecule believed to bind nascent chains of chitin, consequently disrupting its microfibril structure, and presumably weakening the cell wall [29]. Additional genes were identified by screens for mutants possessing altered sensitivity to glucan synthesis inhibitors papulacandin B, aculeacin A, or echinocandin [3,9,10,12,37]. Still other genes involved in cell wall integrity were identified *via* a screen for mutants with altered sensitivity to low osmolarity conditions [34]. Sensor/signaling genes were frequently identified by screening for genes that upon overexpression suppress the phenotypes of mutant strains with aberrant phenotypes [14,17,33,35].

Despite the large number of genes already identified that contribute to yeast cell wall integrity and function, there are likely many more genes that remain to be identified. Recently many genes that presumably have some function related to the cell wall architecture were identified by screening transposon-mutagenized cells for altered sensitivity to calcofluor white [20]. This screen probably was not saturating because most genes were identified only once and there was little overlap with previous screens for calcofluor white mutants, consistent with the notion that additional cell wall genes remain to be identified. There are approximately 2500 yeast ORFs that have no known cellular role [13]. It is likely that many of these ORFs also play a role in cell wall biology. In addition, genes with previously defined cellular roles may have additional roles related to cell wall biology.

Genomic approaches to study yeast biology will greatly facilitate the identification of additional cell wall genes. Knowledge of the DNA sequence of the *S. cerevisiae* genome has had a major impact on the study of yeast biology. This information, which has ushered in the "post genomic era," now permits us to address issues in a more global sense with respect to cell biology. This information can and has been used to further our understanding of the biology of the yeast cell wall; however, it is important to note that these same global approaches are being used in many other areas of research as well. The yeast genome sequence, when combined with excellent scientific collaboration between a number of laboratories, empowered the rapid construction and distribution of yeast deletion strains representing all the predicted yeast ORFs [36]. Preliminary phenotypic studies of these strains have identified many genes related to cell wall function. The identification of physically interacting gene products by the two-hybrid approach is being employed on a genomic scale [31]. Information generated by such a global study will undoubtedly advance our understanding of enzyme complexes and regulatory interactions that impact the cell wall. Furthermore the use of a transposon tagging has allowed for determination of the cellular localization of gene products on a genomic scale [30]. These tags can also be used for mutagenesis and for measuring gene expression.

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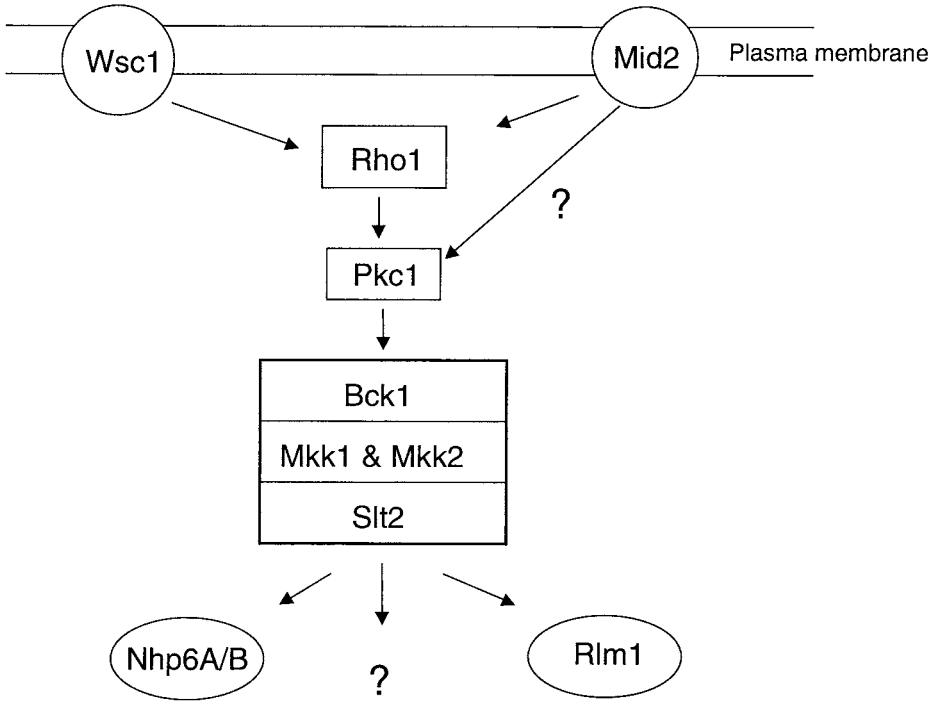

Figure 1 Cell wall integrity pathway. Arrows represent signaling interactions and question marks represent putative interactions or factors.

We have used microarray technology to identify genes involved with cell wall structure/function. Microarrays take advantage of the yeast DNA sequence information and use it to measure quantitatively the global transcription pattern of cells under conditions of interest. Microarrays have been employed to dissect MAP kinase signaling pathways, including the Pkc1 pathway [28]. Such studies were done with the intent to identify genes effected downstream of these pathways and to study how signal transduction pathways impact each other. Furthermore, changes in transcription observed by mutant strains lacking signaling genes or transcription factors are providing insight to downstream regulatory responses [4,7,22,28,32]. We measured the transcription response of yeast cells to multiple forms of cell wall stress to obtain data that upon clustering could be used to identify genes that have a role in cell wall biology. Twenty-five genes responded to hypotonic shock and treatment with the cell wall synthesis inhibitor calcofluor white (Estrem ST and Skatrud PL, unpublished results). Following the identification of these putative cell wall genes, several were further characterized by genetic analysis to determine if the gene products have a role related to the cell wall. As the amount of global transcription data increases, the ability to classify genes of unknown cellular role should be facilitated.

Microarray technology is just one genome-wide approach used to measure gene expression of yeast. Another approach is the global measurement of gene expression at the protein level. Here proteins are separated and quantified by two-dimensional gel electrophoresis and identified by mass spectrometric technology. This approach is advantageous in that mRNA levels do not necessarily correlate with protein levels and important posttranslational modifications that impact protein function can be observed. Yet another approach is to use a library of strains each containing a reporter gene fused to a different gene of the genome. Acacia's Genome Reporter Matrix™ is just such a collection of strains that represents most of the genes encoding proteins in *S. cerevisiae* [8].

A beneficial aspect of global genome-wide expression technology is that there is always the possibility to observe previously unappreciated biological responses. By measuring all possible responding genes to a particular environmental condition, completely unexpected physiological responses deeply embedded in the network of intracellular communication will be uncovered. For example, we observed that phosphate acquisition genes were transcribed greater following hypotonic shock (Estrem ST and Skatrud PL, unpublished results). The induction of these genes may be tied to the decreased synthesis of the osmoprotectants glycerol and trehalose. The synthesis of each provides free phosphate for the cell. Initially these responses may not make much sense, but as our understanding of that complex intracellular communication network matures, the timing and location of the puzzle pieces will become apparent. Clearly, the application of genomics is having a significant impact on our understanding of biology in a manner not imaginable without comprehensive DNA sequence information.

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