The Yeast Cell Wall and Septum as Paradigms of Cell Growth and Morphogenesis*

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Fungal cells, like their bacterial and plant counterparts but unlike animal cells, are encased in a sturdy shell, the cell wall, which is essential for their survival. Fungal cells have a very high turgor pressure, and even a minor chink in the cell wall structure can lead to bursting and death (1). Yet, because the cell wall is external to the cell, it must grow with it and endure all the changes that the cell undergoes during the division cycle. To ensure the continuous integrity of the cell wall without interfering with its plasticity and constant growth, elaborate control mechanisms must be operating, which need to be strictly coordinated with those governing the cell cycle.

There are two major reasons for studying the fungal cell wall and its specialized structure for cell division, the septum. 1) Of theoretical interest is the fact that the cell wall, because it imparts shape to the cell in a constantly changing pattern and because of its relatively simple composition, serves as a good model for morphogenesis at the molecular level. 2) Another reason, of an applied nature, is that cell wall synthesis is an attractive target for antifungal agents because it is unique to the pathogen.

Because of the versatility of budding yeast for biochemical and genetic studies, the most comprehensive body of work on fungal cell walls has been carried out with this organism. Therefore, this review will deal specifically with Saccharomyces cerevisiae.

Composition and Structure of the Yeast Cell Wall

The yeast cell wall consists mainly of polysaccharides made up of three sugars, glucose, mannose, and N-acetylglucosamine. One of the glucose polysaccharides, β(1→3)glucan, is the major structural component of the wall. Another, β(1→6)glucan, is relatively minor in amount but very important for cross-linking (see below). The mannose polysaccharides are linked to proteins to form a mannoprotein layer mainly localized at the external surface and acting as a filter for large molecular weight materials (2). Finally, the small amount of GlcNAc present in the wall (1–3%) is in the β(1→4)-linked linear polysaccharide chitin, which is mostly concentrated in the septal region although some is dispersed throughout the wall. Despite its small quantity, chitin is essential for yeast survival (3), probably because of its central role in septation.

An early indication that the yeast cell wall components are attached to each other by covalent linkages was that mannoproteins, insoluble in native walls, become soluble in water after digestion of the walls with an endo-β(1→3)-glucanase (2). This suggested a direct or indirect attachment between β(1→3)glucan and mannoproteins. The first fully characterized linkage between cell wall components was the β(1→4) bond between the reducing terminal GlcNAc of a chitin chain and the nonreducing terminal glucose of β(1→3)glucan (Fig. 1A) (4). Other studies revealed an association between mannoproteins, β(1→6)glucan and β(1→3)glucan (5). Finally, the linkages of a complex including those three components plus chitin were identified and partially characterized (Fig. 1B) (6). In this complex, β(1→6)glucan occupies a central position. To it, chitin and β(1→3)glucan are directly attached by glycosidic linkages, whereas mannoprotein is joined to the polysaccharide through the lipidless remnant of a glycosylphosphatidylinositol anchor (Fig. 1B). These anchors have been identified as signals for the incorporation of proteins in the cell wall after transit through the plasma membrane (for review, see Ref. 7).

In addition to the complex shown in Fig. 1B, another was isolated that lacked the mannoprotein. Together with the previous identification of a chitin-β(1→3)glucan compound, this finding suggested the notion of a “flexible building block” (6) or “module” (7) for cell wall construction. These modules, of somewhat variable structure, would form a lattice all around the cell, thus generating an essentially continuous envelope. The modules may be joined to each other through hydrogen bonds (Fig. 1C). It is also possible that the β(1→3)glucan branches joined to the main chain by β(1→6) linkages are longer than formerly thought, and this would provide the means for a practically endless network of the branched polysaccharide around the cell (Fig. 1D).

Other linkages are also present in the cell wall. A family of proteins called Pir (proteins with internal repeats) seems to be attached directly to β(1→3)glucan through an alkali-labile linkage of as yet unresolved nature (8, 9). In addition, some proteins are released from the wall with sulfhydryl compounds (Fig. 1, C and D) (10).

Obviously, the cross-links must contribute to the mechanical strength of the wall. They could also be important in the loosening and remodeling of the wall, such as at bud emergence. Some critical linkages could be cut temporarily by appropriate enzymes to allow for wall expansion, while the remainder of the network would keep the wall together.

Biosynthesis of β(1→3)Glucan as a Model for Cell Wall Synthesis and Its Regulation

The growth of the cell wall must accompany that of the cell that it encloses. In particular, new cell wall must be generated at the emergence of a new bud; its synthesis continues throughout the growth of the bud and stops at maturation. To study the temporal and spatial regulatory controls that direct this process, β(1→3)glucan, the major structural component of the yeast cell wall, has been used as a model. The enzymatic system that catalyzes the synthesis of β(1→3)glucan from UDP-glucose is bound to the plasma membrane (for review, see Ref. 11). An early finding was that GTP is a potent activator of β(1→3)glucan synthase at micromolar concentration (11). Later studies with yeast and other fungi led to the stepwise solubilization of two fractions from the membrane, both required simultaneously for glucan synthesis. One contains a small (26 kDa)
Minireview: Yeast Cell Wall and Septum as Paradigms

FIG. 1. Architecture of the yeast cell wall. A, the linkage between chitin and β(1→3)glucan. B, structure of a complex that contains all four components of the cell wall, β(1→3)glucan, β(1→6)glucan, manno-protein, and chitin. Arrows point to reducing ends. P is a phosphate group. C and D, two possible ways of attaching together cell wall modules, either by hydrogen bonds (sets of three black bars in C) or by β(1→3)glucan side chains (D). The color code is the same as in B.

GTP-binding protein; the other harbors the catalytic component(s) of the synthase (11). The GTP-binding protein was identified as Rho1p (12, 13), whereas a large hydrophobic protein, Fks1p, was found in the catalytic fraction (14). Properties of mutants and experiments with specific inhibitors indicate that Fks1p or its close homolog Fks2p is necessary for β(1→3)glucan synthesis in vivo (14). There is no proof that Fks1p or Fks2p contains the catalytic site for glucan synthesis, but they are the only present candidates for such a function. Their participation in the synthetic reaction is supported by the immunoprecipitation of Fks1p and Rho1p (13).

The discovery that a GTP-binding protein was the regulator of β(1→3)glucan synthase went a long way to explain how the synthesis of the polysaccharide product can be started or shut off (11). GTP-binding proteins can act as molecular switches because they are active when bound to GTP and inactive when bound to GDP. The conversion from active to inactive form is stimulated by GTP-pase-activating proteins, whereas the opposite shift is helped by GTP-GDP exchange factors. Two proteins, Rom1p and Rom2p, have been identified as GTP-GDP exchange factors for Rho1p (15), whereas Bem2p and Sac7p are potential, albeit yet unproven, GTP-ase-activating proteins for Rho1p (11).

The shift of Rho1p between the active and inactive state provides a rationale for the switching on and off of β(1→3)glucan and cell wall synthesis. As pointed out below, Rho1p has other functions in addition to regulation of β(1→3)glucan synthase. We recently found that rho1 mutants with a temperature-sensitive defect specific for the glucan synthesis function die at the nonpermissive temperature.1 Although for some time rho1 mutants incorporate other components in the cell wall, these components cannot be anchored to β(1→3)glucan. These results underline the importance of β(1→3)glucan for the preservation of cell wall architecture.

Biosynthesis of Other Cell Wall Components

The mechanism of biosynthesis of β(1→6)glucan, a central component of the wall cross-linked modules, has been studied by a genetic approach. Bussey and co-workers (reviewed in Ref. 16) took advantage of the fact that β(1→6)glucan is a receptor for the KI killer toxin to isolate mutants resistant to the toxin because of their diminished β(1→6)glucan content. Several genes required for synthesis of the polysaccharide were cloned by complementation of the mutations. Based on the sequences of the corresponding proteins, guesses were made about their intracellular localization. From this evidence it was concluded that the synthesis of β(1→6)glucan probably begins in the endoplasmic reticulum, continues in the Golgi, and is completed at the cell surface (16). However, Montijn et al. (17) were unable to find intracellular material reacting with a β(1→6)glucan antibody, even in mutants that accumulated secretory vesicles. Thus, the mechanism of synthesis of β(1→6)glucan remains obscure.

As for mannoproteins, a large amount of information has been built up over the years about the biochemical steps through which oligosaccharides are constructed and transferred to protein in the ER, followed by further elongation in the Golgi (see Refs. 18–21 for reviews). On the other hand, little is known about the mechanism and sites of incorporation of mannoproteins in the cell wall, including the putative transglycosylation reactions by which the glycosylphosphatidylinositol anchors are attached to β(1→6)glucan with elimination of their lipid moiety.

Localization of Cell Wall Growth

In addition to temporal switches of the type involving Rho1p and β(1→3)glucan synthase, spatial controls are required to confine growth of the cell wall to the bud and to direct it in such a way that a bud will be made with the correct shape. These controls are part of the processes of cell polarization and actin cytoskeleton organization, which are beyond the scope of this article (for reviews see Refs. 22 and 23). Suffice it to say that immediately after emergence, the bud grows at the tip (apical growth). Accordingly, Rho1p (13, 24) and Fks1p (13), components of the β(1→3)glucan complex, localize at that site (Fig. 2A). Growth then becomes more uniformly distributed (isotropic growth, Fig. 2B) to generate the ellipsoidal shape of the daughter cell. These changes in growth pattern are accompanied by concomitant changes in the distribution of actin patches (23). Another component of the actin cytoskeleton that is essential for growth, actin filaments, appears to direct into the bud the secretory vesicles that carry cell wall and membrane components as well as enzymes for cell wall synthesis (23).

The Preservation of Cell Wall Integrity

As mentioned above, it is imperative for the cell to maintain cell wall integrity at all times, including stages of the cell cycle involving extensive remodeling of the wall, such as budding and cell division, and situations of stress that may arise in nature, such as extremes of temperature and pH. The cell receives signals of the stressful conditions through a battery of sensors (the proteins Wsc1p/HCS77, Wsc2p, Wsc5p, and Mid2p, at present count) bound to the plasma membrane (25–28). The signal is transmitted somehow to Rho1p (25), which, in addition to its function in β(1→3)glucan synthesis, acts as an activator of the protein kinase C, Pkc1p (29, 30). Pkc1p, in turn, controls a mitogen-activated protein kinase cascade, which is required for cell wall integrity (31). Defects in Pkc1p and in some members of the cascade lead to cell lysis. At least one of the functions of the mitogen-activated protein kinase cascade is as a positive regulator of the transcription of several genes engaged in cell wall biosynthesis, including FKS1 (32). Thus, Rho1 acts on β(1→3)glucan and cell wall formation both directly (as a component of β(1→3)glucan synthase) and indirectly (as an activator of Pkc1p).

1 D.-H. Roh and E. Cabib, unpublished results.
ride chain. Three chitin synthases, CSI, CSII, and CSIII, have been identified in yeast, and the genes believed to code for their catalytic components, CHS1, CHS2, and CHS3, have been cloned (33, 34). The corresponding proteins show a high level of identity in the middle and carboxyl-terminal portion, whereas a nonessential amino-terminal region of variable length is quite dissimilar (38). Several mutations of Chs2p that lead to inactive proteins have been identified (39, 40). The shared motif QRHRW seems to be essential for activity and function of all three synthases (41).

All three chitin synthases are membrane-bound, in part to the plasma membrane and in part to vesicles of uncertain origin that have been named chitosomes (33, 42). A general feature of fungal chitin synthases, shared by CSI and CSII, is that they are found in a mostly inactive state. Partial proteolysis, usually by trypsin, leads to a striking increase in activity (33). The latent form of the synthases has been termed thezymogen. CSIII is isolated in an active state, apparently because an activator, most probably Chs4p, is bound to it (43, 44). Extraction with detergents or mutations in CHS4 revealed a zymogenic form also for CSIII (43). How CSI and CSII are activated in vivo is unknown, but the very existence of the zymogenic forms suggests that activation of the zymogen must be an important step in their regulation.

**Function of Chitin Synthases and Their Regulation**

Examination of the phenotype of mutants in the three chitin synthase genes led to an understanding of their function (3). chs2 mutants are unable to construct a primary septum. Although they usually succeed in completing cell division, their septa are thick, amorphous, and often show lacunae. Chitinase cannot act on these septa, and as a result cell separation is defective and the cells form large clumps. It was concluded that CSI is responsible for the synthesis of primary septum chitin. chs3 mutants do not lay down the chitin ring at the base of emerging buds, but they have a tri-layered septum. They also lack the chitin dispersed in the cell wall and involved in cross-links (4). As for CSI, the defect in this enzyme had a more subtle phenotype; when grown in a medium with poor buffering power, many daughter cells lyse during cell separation with a small hole in the center of the birth scar (1). Chitinase inhibitors reduced the defect and deletion of the chitinase gene abolished it (45). Therefore it appears that CSI has a repair function by providing replacement chitin when the chitinase involved in cell separation (see above) is activated by the decreasing pH of the medium and digests too much of the primary septum.

It is interesting that although single mutants in each one of the three chitin synthase genes, CHS1, CHS2, and CHS3, as well as double mutants chs1 chs2 and chs1 chs3 are viable, simultaneous mutation of CHS2 and CHS3 is lethal (3). The reason for this synthetic lethality is not well understood, but the result indicates that chitin is essential for cell survival and therefore a potential target for antifungals.

How do the three chitin synthases carry out their different tasks? We will start with CSIII because more is known about this synthase. The reason for this resides in the discovery that Calcofluor White, a fluorescent dye that binds to chitin, induces a great increase in the synthesis of the polysaccharide and arrests cell growth. Mutations in CHS3 or other genes required for Chs3p function will result in resistance to Calcofluor. With

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**Fig. 2. Cell wall growth and septum formation.** The cell wall is in gray, and the plasma membrane is the red broken line. In A, CSIII (black), at the base of an emerging bud, has catalyzed the synthesis of the chitin ring (green). Bud growth is in the apical phase (arrow), and Rho1p and Fks1p, components of β1-3glucan synthase, are at the bud tip. B, as the bud enlarges, the growth becomes isotropic (arrows). Rho1p and Fks1p are no longer detectable. Notice in A and B the chitin (green dashes) interspersed in the cell wall of the mother cell but not in the bud. C, a contractile ring (CR) invaginates the plasma membrane at cytokinesis. Concomitantly, CSII (brown) catalyzes the synthesis of primary septum chitin (green). D, the primary septum is now complete and secondary septa (SS, yellow) have been laid down from the mother and daughter cell. Chitin appears in the daughter cell wall. E, cell separation is facilitated by a partial digestion of the primary septum by a chitinase (Chit). CSI (purple) serves as a repair enzyme to provide extra chitin if chitinase action goes too far. BS, bud scar.

**The Septum, a Specialized Structure of the Cell Wall**

At cell division, a cross-wall, the septum, is made between the mother and daughter cell to permit their separation without lysis (reviewed in Refs. 33 and 34). The yeast septum is made in three different stages. The first takes place very early when a chitin ring is formed at the base of an emerging bud (Fig. 2A). The second occurs at cytokinesis, when a contractile actomyosin ring leads to the invagination of the plasma membrane (35, 36). As the membrane invaginates, chitin is secreted from it to form a disc, the primary septum, that is the first structure separating the dividing cells (Fig. 2C). Once the primary septum is completed, the third and last stage starts with the buildup by mother and daughter cells of secondary septa that appear to have a similar composition to the remainder of the cell wall (Fig. 2D). Finally, the two cells are separated by the action of a chitinase (37) that partially hydrolyzes the primary septum (Fig. 2E).

Because of its simple geometrical shape and of its importance for cell division, the septum is an attractive structure for morphogenetic studies. Most such studies have dealt with the biosynthesis of chitin because of the central role of this polysaccharide in septum formation. On the other hand, little is known about the formation of secondary septa, and they will not be discussed here. Chitin synthesis is catalyzed by synthases that transfer GlcNAc from UDP-GlcNAc to a growing polysacchara-
this screen and with another screen based on glucosamine incorporation, CHS3 itself and four other genes, now called CHS4 to CHS7, were cloned (see Ref. 34 for review). One question is how Chs3p reaches the site(s) where it exerts its function. This journey starts in the ER. For export of Chs3p from the ER, another protein, Chs7p, is required (46). The next station is the Golgi, where Chs3p appears to be associated with a different protein, Chs5p (47). Chs5p and Chs6p (48) are necessary for the next step, which is transfer to vesicles (chitosomes) that will carry Chs3p to the plasma membrane (42, 48). However, Chs3p is not accompanied by Chs5p to its final destination, a ringlike area at the base of an emerging bud, where the chitin ring is laid down (42, 47) (Fig. 2A). Chs3p may be retained in this location through its interaction with the putative activator, Chs4p. The latter can bind to Bni4p, which in turn can attach to the septin ring (49), a microfilament ring at the mother cell-bud neck required for cytokinesis and that is supposed to act as a scaffold for many proteins involved in cell division.

For Chs2p and Chs1p there is much less information. A portion of both has been found in a “chitosome” fraction (42, 50). The bulk of these proteins, however, is in the plasma membrane (33). It is interesting that none of the Chs proteins has a signal sequence, although all three use a secretory pathway to reach the membrane.

The regulation of Chs2p is different from that of the other Chs proteins; in that its concentration in the cell changes greatly during the cell cycle, in contrast to the constant level of Chs1p and Chs3p (42, 51). The protein has been located by immunofluorescence at the neck between the mother cell and a large bud (42). This is the time when presumably the zymogen form of the enzyme is activated and construction of the primary septum proceeds (Fig. 2C). Immediately thereafter, Chs2p is transported to the vacuole and destroyed (42). A concomitant sharp decrease in its level was detected (42, 51).

Concluding Remarks

The cell wall and septum together make up about one-third of the yeast cell dry weight. Thus, the cell devotes a great deal of its energy and of its synthetic apparatus to generate these structures. Moreover, cell wall and septum construction is part of cell growth and division; therefore, it must be strictly regulated in space and time to accompany the other processes that are part of the cell cycle. By studying those regulatory mechanisms we learn how the cell localizes proteins, activates enzymes, and builds structures with a designed shape. Thus, the study of yeast cell wall and septum formation has provided a rich harvest of knowledge about fundamental cellular processes. At the same time, new targets for antifungal compounds have been defined. The main ones so far have been chitin and β1–3)glucan synthesis, for which inhibitors, such as the polyoxins and nikkomycins for chitin and the semisynthetic echinocandins for glucan, have become available. The glucan synthesis inhibitors are now undergoing clinical tests. Further study of the fungal cell wall may be expected to continue to furnish both exciting new findings and clinical applications.

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