Cell Wall-Related Bionumbers and Bioestimates of Saccharomyces cerevisiae and Candida albicans.

Klis, F.M.; de Koster, C.G.; Brul, S.

DOI
10.1128/EC.00250-13

Publication date
2014

Document Version
Final published version

Published in
Eukaryotic Cell

EC Journals.ASM.org

MINIREVIEW

Cell Wall-Related Bionumbers and Bioestimates of *Saccharomyces cerevisiae* and *Candida albicans*

Frans M. Klis, Chris G. de Koster, Stanley Brul

Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

Bionumbers and bioestimates are valuable tools in biological research. Here we focus on cell wall-related bionumbers and bioestimates of the budding yeast *Saccharomyces cerevisiae* and the polymorphic, pathogenic fungus *Candida albicans*. We discuss the linear relationship between cell size and cell ploidy, the correlation between cell size and specific growth rate, the effect of turgor pressure on cell size, and the reason why using fixed cells for measuring cellular dimensions can result in serious underestimation of *in vivo* values. We further consider the evidence that individual buds and hyphae grow linearly and that exponential growth of the population results from regular formation of new daughter cells and regular hyphal branching. Our calculations show that hyphal growth allows *C. albicans* to cover much larger distances per unit of time than the yeast mode of growth and that this is accompanied by strongly increased surface expansion rates. We therefore predict that the transcript levels of genes involved in wall formation increase during hyphal growth. Interestingly, wall proteins and polysaccharides seem barely, if at all, subject to turnover and replacement. A general lesson is how strongly most bionumbers and bioestimates depend on environmental conditions and genetic background, thus reemphasizing the importance of well-defined and carefully chosen culture conditions and experimental approaches. Finally, we propose that the numbers and estimates described here offer a solid starting point for similar studies of other cell compartments and other yeast species.

In their paper "A feeling for the numbers in biology," Phillips and Milo (1) present a convincing case for a more quantitative approach in biological research. An important advantage of moving from a qualitative to a more quantitative understanding of a biological process is that one learns to view one's observations from a different perspective. A feeling for the numbers involved assists in prioritizing hypotheses and selecting better experimental approaches and leads to surprising insights. For industrial purposes, genetic engineering, and synthetic biology, a quantitative approach becomes even more important, whereas for modeling of biological processes, accurate bionumbers and bioestimates are crucial. Numerous interesting bionumbers can be found at the website http://bionumbers.hms.harvard.edu/ (2). The cell wall of yeasts accounts for up to 30% of the cellular biomass on a dry weight basis and thus represents a substantial metabolic investment of the cell. Here we focus on cell wall-related bionumbers and bioestimates of two important fungi: the workhorse *Saccharomyces cerevisiae* and the human pathogen *Candida albicans*.

CELLULAR DIMENSIONS OF *SACCHAROMYCES CEREVISIAE*

The shape of the parent cell and the growing bud of the yeast *S. cerevisiae* (and of *C. albicans* when growing in the yeast form) approximates a prolate ellipsoid. This allows accurate estimation of the volume and surface area of parent cell and bud by measuring their length (major axis) and width (minor axis). For example, the volume $V = \pi ab^2/6$ (where a is the major and b is the minor axis) and is usually expressed in μm^3 or fl ($1\ \mu m^3 = 1\ fl = 10^{-15}$ liter). The online calculator "Ellipsoid" at http://planetcalc.com/149/ allows rapid calculation of both volume and surface area of ellipsoids.

The cellular dimensions of parent cells of *S. cerevisiae*, which is usually grown at 30°C, have been extensively investigated. They vary widely and depend on cell ploidy, growth rate, and nutrient status and also on turgor pressure and the number of buds formed by the parent cell. Table 1 shows that in exponential-phase cultures growing in rich medium, the average volume of parent cells is proportional to ploidy, increasing from ~44 μm^3 for haploid cells to ~244 μm^3 for hexaploid cells (3). This agrees with the median values of 42 μm^3 and 82 μm^3 obtained with haploid and diploid cells, respectively (4); see also reference 5, in which average volumes of 56 and 95 μm^3 are presented for haploid and diploid cells, respectively. Table 1 further shows that the ratio of surface area and volume is inversely correlated with cell ploidy, decreasing by ~40% (from 1.38 μm^{-1} for haploid cells to 0.79 μm^{-1} for hexaploid cells). This is consistent with the observation that in diploid cells the transcript levels of genes that are involved in cell wall formation are generally lower than in haploid cells (6).

The mean volume of parent cells of *S. cerevisiae* not only increases with increasing ploidy but, as has been shown for diploid cells, also positively correlates with the specific growth rate (Table 2), increasing from 29 μm^3 at a specific growth rate of 0.045 h^{-1} (doubling time of ~15 h) to 95 μm^3 at 0.46 h^{-1} (doubling time of ~1.5 h) (5, 7); a similar trend has been observed for haploid cells (8, 9). Interestingly, reduced growth resulting from either nutrient limitation or gene mutation is accompanied not only by a decrease in cellular volume but also by increased tolerance toward various stress conditions such as heat and oxidative stress (10). Similarly, post-exponential-phase yeast cells become rapidly more resistant to the wall-degrading enzyme preparation Zymolyase (11). This raises the question of whether nutrient sensing pathways, such as the Ras-cyclic AMP-protein kinase A signaling pathway and the Snf1 and TORC1 pathways (12), control specific cell wall properties, depending on nutrient availability. Finally, the inverse rela-

Published ahead of print 15 November 2013

Address correspondence to Frans M. Klis, F.M.Klis@uva.nl.

doi:10.1128/EC.00250-13

TABLE 1 Effect of cell ploidy on dimensions, volume, biomass, surface area, and the ratio of surface area over volume of *S. cerevisiae* parent cells[a]

Ploidy	Length (μm)	Width (μm)	Vol (μm^3)	Biomass (dry wt, pg)	Surface area (μm^2)	Surface/vol (μm^{-1})
1	4.76	4.18	44	16.5	60	1.38
2	6.16	5.06	83	31.2	92	1.12
3	7.66	5.97	143	53.9	134	0.93
4	7.97	6.20	161	60.5	144	0.90
5	9.42	6.64	218	82.1	179	0.82
6	10.1	6.80	245	92.3	194	0.79

[a] This table is based on data from plate-grown cells (3). The biomass (dry weight) was calculated by multiplying the volume with the density (1.11) (88) to obtain the biomass (wet weight) of the cell and multiplying the obtained value with the dry weight fraction (0.34) of the wet weight (89).

TABLE 3 Macromolecular compositions of the walls of haploid yeast cells of *S. cerevisiae* and diploid yeast cells of *C. albicans* grown in rich medium at 30°C (62)

Component	*S. cerevisiae*[a]	*C. albicans*[a]
Mannoprotein		
Protein (%)	4.0	3.5
Mannan (%)	34.2	26.6
Mannan/protein ratio	8.6	7.6
β-Glucan[b] (%)	60.3	64.0
β-1,3-Glucan/β-1,6-glucan ratio	3.8	2.5
Chitin (%)	1.4	4.2

[a] The wall dry weight of *S. cerevisiae* is ~21% of the total biomass (67). For *C. albicans* yeast cells, the same value is assumed.
[b] β-1,3-Glucan plus β-1,6-glucan.

tionship between surface area and cell volume probably favors the use of small (haploid) cells in cell surface engineering when the primary goal is to maximize the number of surface-located heterologous proteins per unit of biomass. Conversely, the use of large (polyploid) cells is probably preferred when one is interested in maximizing intracellular contents.

Turgor pressure is another, often overlooked factor that can strongly affect cell dimensions. The turgor pressure in exponential-phase yeast cells cultured under hypo-osmotic conditions is estimated to be ~0.6 MPa (13), similar to the tire pressure of racing bikes. Yeast and hyphal walls are elastic and, owing to the turgor pressure exerted on the walls when the cells are cultured in hypo-osmotic media, do expand considerably, resulting in turgid cells (13). When yeast cells are transferred to hyperosmotic solutions, their volume immediately decreases by 40 to 60% (14, 15). Consistent with this, cells grown in media of increasing osmotic strength become smaller and the porosity of the wall decreases (16). Cell contraction in a hyperosmotic medium is reversible: when the cells are transferred back to hypo-osmotic conditions, they rapidly regain their original volume (14). Cell contraction in hyperosmotic solutions has also been observed in several other yeasts, including *Candida* spp., the fission yeast *Schizosaccharomyces pombe*, and the basidiomycetous yeast *Cryptococcus laurentii* (14, 17), indicating that cell wall extension caused by turgor pressure is a general phenomenon. Cells contract also during fixation. For example, the volume of *S. cerevisiae* yeast cells decreases overnight by about 30% upon fixation in buffered glutaraldehyde (18). This means that volume measurements based on fixed cells (19, 20) tend to (seriously) underestimate the original volume. These observations raise the intriguing question of whether the invaginations of the plasma membrane that are often observed in electron microscopy pictures might be (partially) attributed to inward folding of the plasma membrane that becomes too wide for the cell owing to cell contraction during fixation and subsequent processing steps. Finally, the volume of parent cells has been shown to increase during each budding cycle by about 20% (21–23).

TABLE 2 Cellular volume of diploid *S. cerevisiae* parent cells is positively correlated with the specific growth rate[a]

Specific growth rate (h^{-1})	Vol (μm^3)	Biomass (dry wt, pg)
0.045	29	11
0.10	31	12
0.15	35	13
0.25	48	18
0.30	61	23
0.35	69	26
0.46	95	36

[a] The first six data points are from reference 7, whereas the last point is from reference 5. Biomass (dry weight) was calculated as described for Table 1.

CELLULAR DIMENSIONS OF *CANDIDA ALBICANS*

C. albicans is a polymorphic fungus that can grow as budding yeast and as pseudohyphae, but it can also form authentic hyphae with an apical body and perforated septa (24, 25). It mainly occurs as diploid cells, but haploid and tetraploid cells have also been observed (26). As *C. albicans* is associated with warm-blooded animals, we preferentially use bionumbers obtained at 37°C. Similar to those of *S. cerevisiae* yeast cells, the cellular dimensions of *C. albicans* yeast cells vary widely (20). Although fixed cells were used in this study, the trends were similar to those obtained with *S. cerevisiae* cells, including a positive correlation between cell size and growth rate and a slight increase in parental cell volume with each budding cycle (20). Measurements of live, exponential-phase parent yeast cells grown in a defined salt medium at 25°C resulted in an average calculated volume of 88 μm^3 (27) and in a slightly higher volume, 95 μm^3, when the cells originated from an exponential-phase culture grown in rich medium at 37°C (28). Both values are similar to the values obtained with *S. cerevisiae* diploid parent cells originating from an exponential-phase culture grown in rich medium (3–5). Hyphal width of *C. albicans* can also vary widely (19). Under optimal growth conditions, a diameter of 2.6 μm has been observed for germ tubes and a diameter of 3.4 μm for mature hyphae (29).

QUANTITATIVE ANALYSIS OF THE CELL WALLS OF *S. CEREVISIAE* AND *C. ALBICANS*

The general compositions of the walls of *S. cerevisiae* and *C. albicans* yeast cells are similar (Table 3). On a more detailed level, however, substantial differences emerge. For example, their wall proteomes differ sharply and the *N*-linked carbohydrate side chains of mannoproteins of *C. albicans* contain β-mannosides (30), which are lacking in *S. cerevisiae*. The molecular organizations of the walls of both species, including hyphal walls of *C. albicans*, are also similar (31, 32). Their walls consist of an external mannoprotein coat shielding an inner polysaccharide network that consists of β-glucans and chitin and is responsible for the mechanical strength of the wall. It has been proposed that the external protein coat consists of a basal layer of small, glycosylphosphatidylinositol (GPI)-modified wall proteins interlinked by

disulfide bridges that is interspersed with much longer, fibrillar GPI-modified proteins (31, 33) that measure up to ~150 nm in the case of *C. albicans* (17). This could explain why yeast walls are sometimes described as having a three-layered structure (34, 35). A similar three-layered wall structure has been described for *Exophiala dermatitidis* (36, 37). For a more detailed description of the molecular architecture of the walls of both *S. cerevisiae* and *C. albicans* and their wall proteomes, the reader is referred to recent reviews (31–34, 38, 39).

The exact cell wall composition can vary considerably (32, 40). The cell rapidly responds to changing environmental conditions by adapting the composition of the wall proteome of newly formed walls, by changing the thickness of the skeletal layer of the new walls (17, 40), and by varying the incorporation of chitin (32, 38, 41, 42). There is no clear evidence that the composition of existing walls is drastically changed in such cases, except probably for increased chitin incorporation upon cell surface stress (32). The wall proteome of *C. albicans* has been extensively studied both qualitatively and quantitatively (39, 43). It shows an amazing adaptability that helps the cell to adequately respond to changes in, for example, pH, temperature, hypoxic conditions, carbon source, and iron availability and to plasma membrane and wall stress (43–49). The wall proteome also reflects morphotype and contains yeast- and hypha-specific wall proteins (50). In addition, the wall proteome is involved in controlling cell wall porosity and cell adhesiveness and hydrophobicity, n recognizing specific substrates, and in forming biofilms (11, 16, 17, 39, 49, 51).

PHYSICAL PROPERTIES OF THE CELL WALL

Wall thickness varies considerably. Transmission electron micrographs suggest that the variations in thickness of the walls are largely due to variable thickness of the internal skeletal layer. Although a sharp demarcation is not always present between the inner and outer wall layers, the inner polysaccharide layer can usually be recognized because it is more electron transparent than the outer protein layer. When *C. albicans* yeast cells are transferred to a hyphal-growth-inducing medium, one or more germ tubes emerge from the parent cell. Interestingly, the wall of the emerging hyphae is considerably thinner than the walls of the parent cell (52). However, it is unknown if the thinner wall of the emerging hypha is an inherent quality of hyphal walls or resulted from the composition of the hyphal-growth-inducing medium used in this study. The dependence of the thickness of the inner transparent layer of the walls of *C. albicans* yeast cells on medium composition is illustrated by the observation that the thickness of the inner layer decreases from 75 nm to about 22 nm when the cells are transferred from glucose to the much poorer carbon source lactate (17). Recently, atomic force microscopy (AFM) measurements of wall thickness of live, haploid cells of *S. cerevisiae* have been introduced (53). Dupres and coworkers used a functionalized AFM tip, allowing it to bind to a carboxy-terminal His tag of plasma membrane-bound, elongated forms of the sensor protein Wsc1. As the external protein layer seems to consist of a basal layer of short GPI proteins interspersed with long, fibrillar proteins radiating from the cell surface, it is conceivable that the AFM tip can penetrate the protein layer to a considerable extent and recognize the His tag as soon as it becomes part of or emerges above the basal layer of the mannoprotein coat. This suggests that this method results in values that are mainly determined by the thickness of the inner skeletal layer. AFM measurements of the wall thickness of haploid, late-exponential-phase cells of *S. cerevisiae* resulted in values of about 115 nm (53). This corresponds closely to the electron microscopy-based estimation of the inner skeletal layer of haploid exponential-phase cells equaling ~120 nm (54); interestingly, the inner transparent layer of stationary-phase cells of *S. cerevisiae* was about twice as high (54).

Atomic force microscopy also allows measuring of an important mechanical property of the walls of live cells, i.e., the elastic modulus (E), or Young's modulus. Young's modulus is defined as "stress/strain" and reflects the stiffness of a material; it has units of pressure and is often expressed in MPa. With haploid, glucose-grown, exponential-phase yeast cells of *S. cerevisiae* (55) and diploid yeast cells of *C. albicans* (17), a value of 1.6 MPa was obtained. The Young's modulus of haploid, stationary-phase *S. cerevisiae* cells was also 1.6 MPa (55). Dague and coworkers also included haploid wall mutants of *S. cerevisiae* in their study. For example, the walls of *gas1* cells, which lack a GPI-modified protein involved in the processing of β-1,3-glucan (56, 57), and of *crh1 crh2* cells, which lack two related chitin transglycosylases (57), show a strongly decreased stiffness ($E = 0.80$ and 0.26 MPa, respectively). The carbon source can strongly affect cell wall stiffness as well; for example, the Young's modulus of walls of *C. albicans* yeast cells grown on lactate as the sole carbon source increased more than 3-fold to 5.3 MPa (17). These two examples of the use of AFM in analyzing cell surface properties illustrate that it is a powerful technique to measure physical properties of the wall; for a recent review of how AFM can be used to measure other cell surface properties on a nanoscale level, the reader is referred to reference 58.

Another physical property of cell walls is wall porosity. Early measurements of wall porosity of live cells of *S. cerevisiae* and *C. albicans* have been carried out with stationary-phase cells. In both organisms, only small polyethylene molecules (*S. cerevisiae*, $M_r \leq 760$, hydrodynamic radius ≤ 0.89 nm; *C. albicans*, $M_r \leq 620$, hydrodynamic radius ≤ 0.81 nm) could penetrate the cell wall (59, 60). However, exponential-phase *S. cerevisiae* cells can internalize 70-kDa dextrans (but not 250-kDa dextrans) (61), suggesting that walls of exponential-phase cells have much wider pores than stationary-phase cells. This was confirmed by De Nobel and coworkers, who introduced a rapid assay for relative measurements of wall porosity in live *S. cerevisiae* cells and other yeasts based on the cell lytic activity of the polycation DEAE-dextran (16). Not only is the porosity of the walls of live, exponential-phase cells grown in rich medium indeed much higher than in stationary-phase cells (11), but also it is much higher than the wall porosity of cells growing in minimal medium (16). Interestingly, cell wall porosity increased strongly after pretreatment of the cells with the reducing agent dithiothreitol, pointing to an important contribution by (presumably) intermolecular disulfide bridges between wall proteins in limiting wall porosity (15). Recently, Ene et al. (17), using the same assay, showed that lactate-grown *C. albicans* had a 2-fold higher relative cell wall porosity than glucose-grown cells. In summary, the earlier measurements of wall porosity expressed as exclusion limits in daltons or maximal hydrodynamic radius of the penetrating molecules are probably correct but are valid only for stationary-phase cells, whereas the walls of exponential-phase cells are much more porous depending on growth conditions, although exact values of the corresponding maximal hydrodynamic radii are currently unknown.

TABLE 4 Cell wall-related bionumbers and bioestimates of live, haploid, exponential-phase parent cells and budding cells of *S. cerevisiae* grown in rich medium at 30°C

Parameter	Value(s)	Reference
Haploid parent yeast cells		
Length and width	4.76 and 4.18 μm	3
Surface area	60.0 μm^2	
Vol	43.6 μm^3	
Density	1.11 g cm^{-3} (= pg μm^{-3})	88
Biomass (wet wt)	48.4 pg	
Biomass (dry wt)	16.5 pg (34% of wet wt)	89
Cellular protein	8.7 pg (53% of dry wt)	74
Walls	3.5 pg (21% of dry wt)	67
Wall protein	0.14 pg (4% of walls)	62
β-Glucan	7.7×10^9 Glc	62
β-1,3-Glucan	6.1×10^9 Glc	62
	3.9×10^6 molecules[a]	62
β-1,6-Glucan	1.6×10^9 Glc	
	6.6×10^6 molecules[b]	
Cellular proteins[c]	1.0×10^8	
Wall proteins		
No.[c]	1.6×10^6	
Surface density	2.7×10^4 μm^{-2}	
Haploid budding cells		
Biomass formation	0.18 pg min^{-1}; dry wt	
Surface expansion rate[d]	0.67 μm^2 min^{-1}	
Incorporation rate in bud walls[d]		
β-1,3-Glucan	6.8×10^7 Glc min^{-1}	
β-1,6-Glucan	1.8×10^7 Glc min^{-1}	
Wall proteins	1.8×10^4 min^{-1}	

[a] Based on an estimated length of 1570 glucose residues (90).
[b] Based on an estimated length of 247 glucose residues (91).
[c] Based on an average protein mass of 52,728 (466 amino acids) (2; BN105224).
[d] Based on a generation time of 90 min and linear growth during that period.

TABLE 5 Cell wall-related bionumbers and bioestimates of live, diploid, exponential-phase parent yeast cells, budding cells, and hyphae of *C. albicans* grown in rich medium at 37°C

Parameter	Value(s)	Reference
Diploid parent yeast cells		
Length and width	6.0 and 5.3 μm	27
Surface area	96.1 μm^2	
Vol	88.2 μm^3	
Density[a]	1.11 g cm^{-3} (= pg μm^{-3})	88
Biomass (wet wt)	98.0 pg	
Biomass (dry wt)[a]	33.3 pg (34% of wet wt)	89
Cellular protein[a]	17.7 pg (53% of dry wt)	74
Walls[a]	7.0 pg (21% of dry wt)	67
Wall protein	0.24 pg (3.5% of walls)	62
β-Glucan	1.6×10^{10} Glc	62
β-1,3-Glucan	1.1×10^{10} Glc	62
β-1,6-Glucan	4.8×10^9 Glc	62
Cellular proteins[b]	2.0×10^8	
Wall proteins		
No.[b]	2.9×10^6	
Surface density	3.0×10^4 μm^{-2}	
Diploid budding cells		
Biomass formation[c]	0.37 pg min^{-1}; dry wt	
Surface expansion rate[c]	1.1 μm^2 min^{-1}	
Incorporation rate in bud walls[c]		
β-1,3-Glucan	8.5×10^7 Glc min^{-1}	
β-1,6-Glucan[d]	4.3×10^7 Glc min^{-1}	
Wall proteins	3.2×10^4 min^{-1}	
Elongating hyphae		
Diam		
Germ tubes	2.6 μm	29
Mature hyphae	3.4 μm	29
Elongation rate		
Germ tubes	0.32 μm min^{-1}	29
Mature hyphae	0.76 μm min^{-1}	29
Surface expansion rate[e]		
Germ tubes	2.6 μm^2 min^{-1}	29
Mature hyphae	8.1 μm^2 min^{-1}	29
Wall proteins		
Surface density	2.8×10^4 μm^{-2}	
Incorporation rate		
Germ tubes	7.3×10^4 min^{-1}	
Mature hyphae	2.3×10^5 min^{-1}	

[a] Assumed to be equal to the value obtained for *S. cerevisiae* (88, 89).
[b] Based on an average protein mass of 52,728 (466 amino acids) as determined for *S. cerevisiae* (2) (BN105224).
[c] Based on a generation time of 90 min and linear growth during that period.
[d] Based on the ratios of β-1,3-glucan and β-1,3-glucan as determined in reference 62.
[e] Surface expansion rate (μm^2 min^{-1}) = π × diameter × (hyphal elongation rate).

QUANTITATIVE ANALYSIS OF THE WALL PROTEOME

The polypeptide part of the walls of both *S. cerevisiae* and *C. albicans* accounts for ~4% and 3.5% of the wall dry weight, respectively (35, 62) (Table 3). In view of the high mannose content of the walls, this indicates that their wall proteins are generally highly glycosylated. It seems likely that in particular very small GPI-modified, N-glycosylated wall proteins such as ScCcw12 (63) and CaPga59 and CaPga62 (35) contribute to this high mannose content. Their corresponding genes each have an exceptionally high codon adaptation index (0.87, 0.95, and 0.91, respectively; *Saccharomyces* Genome Database at http://www.yeastgenome.org/ and *Candida* Genome Database at http://www.candidagenome.org/) and thus probably encode highly abundant proteins (64).

The total number of wall proteins in a haploid parent cell of *S. cerevisiae* from an exponential-phase culture growing in rich medium is estimated to be ~1.6×10^6 (based on an average molecular mass of 52,728 Da) (2) (http://bionumbers.hms.harvard.edu/; BN 105224), which corresponds to a surface density of about 2.7×10^4 μm^{-2} (Table 4). For diploid *C. albicans* yeast cells the corresponding estimates are 2.9×10^6 wall proteins per parent cell and a surface density of 3.0×10^4 μm^{-2} (Table 5). The minimal radius of a spherical 53-kDa protein equals 2.48 nm (65). Efficient (hexagonal) packing of such proteins on a surface would then result in a surface density of ~$4.7 \times^4$ μm^{-2}. This suggests that the outer protein layer of the wall can be approximately viewed as a monolayer, especially when taking into consideration that many wall proteins carry bulky *N*-linked carbohydrate side chains, resulting in increased protein dimensions and thus a lower surface density. The wall proteomes of both *S. cerevisiae* and *C. albicans* consist of >20 different wall proteins under any growth condition studied. These are not necessarily uniformly distributed over the cell wall, as several wall proteins are known to be cell cycle regulated (66). To obtain absolute copy numbers of individual wall proteins in *S. cerevisiae*, Yin and coworkers (67) used the iTRAQ (isobaric tags for relative and absolute quantitation) reagents (68) for isobaric tagging of tryptic peptides originating from wall pro-

teins on the one hand and synthetic peptides that were added as internal standard on the other hand. Currently, the absolute copy numbers of five cell wall proteins in haploid, exponential-phase cells growing in rich medium have been determined (67): (i) the GPI-modified protein Cwp1, which localizes specifically to the birth scar (66), 6.7×10^4 copies per parent cell; (ii) the GPI-modified protein Crh1, which is a chitin transglycosylase, 3.9×10^4 copies per parent cell; (iii) the non-GPI-modified wall protein Scw4 and predicted β-glucanase, 3.4×10^4 copies per parent cell; (iv) the GPI-modified protein Gas1, a β-glucan transglycosylase, a major plasma membrane protein but also present in the wall, 1.0×10^4 copies per parent cell; and (v) the GPI-modified protein Ecm33, a plasma membrane protein involved in an unknown way in cell wall construction but also consistently identified in the wall, 5.8×10^3 copies per parent cell (67); note that except for Cwp1, which is targeted to the birth scar, the original numbers were slightly corrected, using a correction factor of 0.89, to compensate for the fact that in the original paper the presence of buds was not taken into account. The correction factor was obtained as the ratio of the calculated dry weight of a haploid parent cell (16.5 pg [Table 4]) and the dry weight of a cellular unit in a haploid budding culture (18.6 pg) (67).

QUANTITATIVE ANALYSIS OF THE SECRETOME

The mannoproteins of the wall (and the β-glucans and chitin) of exponential-phase cells of *S. cerevisiae* are metabolically stable and show only limited turnover or replacement (69, 70). This is consistent with the observations obtained with fluorescein-conjugated concanavalin A to label existing mannoproteins. Incorporation of new and thus unlabeled wall proteins took mainly place in the distal tip of the growing bud (71). Although some release of mannoproteins into the medium was observed (69), this is probably largely attributable to local dissolution of the wall in the neck region to allow cell separation (note that before cell separation, the walls of the parent cell and the bud form a continuous structure). These earlier observations are supported by analysis of the secretome of *C. albicans* (72, 73). The absolute amount of proteins recovered by filtration over a 10-kDa filter from the culture medium of late-exponential-phase yeast cells was relatively small (72): ~0.8 mg (g biomass; dry weight)$^{-1}$, or ~1.5 mg/g cellular protein, assuming that cellular proteins account for about 53% of the total biomass (74) (Tables 4 and 5). Mass spectrometric analysis of the medium proteins demonstrated that the majority of them (33 proteins) had a predicted extracellular location and included most of the experimentally identified wall proteins, whereas 13 predicted, cytosolic proteins were identified (72). As wall proteins in *C. albicans* yeast cells are estimated to account for ~14 mg/g cellular protein (Table 5), it is clear that release of wall proteins either by turnover and replacement or by local enzymatic dissolution of the wall during cell separation is limited (<1.5/14, or <11%). A convincing example of wall protein stability in *C. albicans* is the GPI-modified adhesion protein Als1, which after its early, but temporary, incorporation into yeast or hyphal walls persists long into stationary phase (75). Finally, it has been proposed that transwall transport of vesicles plays an important role in fungal physiology (76). However, the relatively small amount of cytosolic proteins found in the medium of *C. albicans* (<0.15% of the total amount of cellular protein) under the growth conditions used suggests that if such an active mechanism exists in *C. albicans*, its role is limited (49, 72). Conceivably, the membranous vesicles isolated from the medium of *C. albicans* (74) originated from apoptotic or dead cells. Nevertheless, from a diagnostic point of view it is a relevant observation.

GROWTH RATES AND SURFACE EXPANSION RATES OF *S. CEREVISIAE* AND *C. ALBICANS*

Population growth can be accurately approximated by an exponential function: $B_t = B_0 \times e^{kt}$ where B is biomass, t is time, and k is the specific (relative) growth rate, which is usually expressed in reciprocal hours (h^{-1}) or reciprocal min (min^{-1}); one also frequently encounters the doubling time, which is related to the specific growth rate by the following equation: (doubling time) $\times k =$ ln 2. However, the biomass (dry weight) of individual budding cells of *S. cerevisiae* and the volume of individual *C. albicans* yeast cells increase linearly over at least the main part of the cell cycle (77, 78). This is further supported by the observation that the surface area of growing buds of diploid *S. cerevisiae* cells increases linearly over a large period with a constant surface expansion rate of 0.75 μm^2 min^{-1} at 25°C and 1.17 μm^2 min^{-1} at 37°C (79). Hyphae of *C. albicans* also elongate with a constant rate, resulting in a linear increase of the surface area in time (28, 29) similar to mycelial fungi (80); note, however, that on a more detailed timescale the elongation rates of the latter oscillate (80). Exponential growth of the population is thus primarily obtained by regular bud formation and hyphal branching or a combination of both (29). Assuming linear growth of individual cells and a doubling time of 90 min, biomass formation (dry weight) by a budding haploid cell of *S. cerevisiae* with an average volume of ~44 μm^3 equals 0.18 pg min^{-1} on a dry weight basis (Tables 1 and 4), whereas biomass formation (dry weight) of a diploid budding cell with a volume of 83 μm^3 (3) is 0.34 pg min^{-1}. For budding diploid yeast cells of *C. albicans* with a volume of 88 μm^3 (25) biomass formation (dry weight) is estimated to be ~0.37 pg min^{-1} (Table 5).

The hyphal elongation rate of *C. albicans* depends on growth conditions and can reach values up to ~46 μm h^{-1} (Table 5). Although hyphae of many filamentous fungi can elongate considerably faster (81), the elongation rate of hyphae of *C. albicans* is much higher than that of yeast cells. A single yeast cell has under favorable growth conditions an average length of ~6 μm, which corresponds to a length increase of only ~4 μm h^{-1}. This shows that hyphal growth permits *C. albicans* to cover much larger distances per unit of time than the yeast mode of growth, thus promoting tissue invasion and biofilm outgrowth, which both imply hyphal growth. Conceivably, the accelerated extension rate of hyphae versus yeast cells also facilitates the escape of *C. albicans* cells from macrophages upon their internalization and subsequent switch from yeast to hyphal growth (82). The accelerated extension rate associated with hyphal growth is not necessarily accompanied by a similar increase in the production of intracellular proteins because the subapical compartments of the hyphae of *C. albicans* tend to become strongly vacuolated (28). Surface expansion rates at 37°C of *C. albicans* yeast cells are ~1.1 μm^2 min^{-1} (Table 5). During hyphal growth of *C. albicans* the surface expansion rates increase considerably to ~2.6 μm^2 min^{-1} for germ tubes and ~8.1 μm^2 min^{-1} for mature hyphae (29) (Table 5), suggesting that wall protein synthesis in growing hyphae is much more prominent than in yeast cells. Table 5 shows that the estimated incorporation rate of wall proteins in *C. albicans* increases from 3.2×10^4 min^{-1} in yeast cells to 7.3×10^4 min^{-1} in germ tubes (a 2.3-fold increase) and to 2.3×10^5 min^{-1} in mature

hyphae (a 7.2-fold increase). It seems likely that hyphal growth implies increased expression levels not only of wall protein-encoding genes but probably also of other genes involved in cell wall formation.

CELL WALL-ASSOCIATED BIONUMBERS AND BIOESTIMATES

Tables 4 and 5 present an overview of cell wall-associated measurements and related bioestimates of live, haploid, exponential-phase parent cells and budding cells of *S. cerevisiae* growing in rich medium at 30°C, and live, diploid, exponential-phase parent yeast cells (and budding cells) and exponential-phase hyphae of *C. albicans* growing in rich medium at either 30 or 37°C. Many questions with regard to cell wall synthesis remain unanswered or have only been addressed in a qualitative way. For example, how is cell wall synthesis adjusted to (i) changes in cell volume (and thus to changes in surface/volume ratios) that accompany changes in cell ploidy and growth rate; (ii) changes in morphotype, for example, upon the switch from yeast to hyphal growth in *C. albicans*; (iii) changes in medium composition and environmental conditions; or (iv) various stress conditions (12, 31, 41, 83)? We hope that the bionumbers and bioestimates discussed here will lead to a more precise understanding of these processes. The approach taken in this review will also be useful for the analysis of other cellular parts or compartments such as the plasma membrane or the cytoplasm. For example, assuming that the wall of haploid, exponential-phase yeast cells is 120 nm thick (53) and adjusting the cellular dimensions of live cells (Table 4) for this, the plasma membrane has a calculated surface area of 54 μm^2. In combination with an estimated 1.26×10^6 Pma1 molecules per cell (64), this results in a surface density of $\sim 2 \times 10^4\ \mu m^{-2}$. Considering that Pma1 is an integral membrane protein with 10 predicted transmembrane domains, this indicates that the plasma membrane is packed with Pma1 (84). Our data can further be used as a starting point for a more quantitative analysis of other yeasts such as *Candida glabrata* (62) and other *Candida* spp., the industrial yeast *Kluyveromyces lactis* (85), and the fission yeast *Schizosaccharomyces pombe* (86, 87). Interestingly, the haploid yeast form of *E. dermatitidis* (exponential-phase cells growing in rich medium) has been studied in depth by serial sectioning followed by a quantitative three-dimensional structural analysis (36, 37). Although these two studies used freeze-substituted cells, presumably resulting in cell contraction, many of their measurements, such as the number of ribosomes/cell ($\sim 2 \times 10^5$) (36), probably predict the corresponding values in haploid, exponential-phase *S. cerevisiae* cells reasonably well.

REFERENCES

1. **Phillips R, Milo R.** 2009. A feeling for the numbers in biology. Proc. Natl. Acad. Sci. U. S. A. **106:**21465–21471. http://dx.doi.org/10.1073/pnas.0907732106.
2. **Milo R, Jorgensen P, Moran U, Weber G, Springer M.** 2010. BioNumbers—the database of key numbers in molecular and cell biology. Nucleic Acids Res. **38:**D750–D753. http://dx.doi.org/10.1093/nar/gkp889.
3. **Mortimer RK.** 1958. Radiobiological and genetic studies on a polyploid series (haploid to hexaploid) of *Saccharomyces cerevisiae*. Radiat. Res. **9:**312–326. http://dx.doi.org/10.2307/3570795.
4. **Jorgensen P, Nishikawa JL, Breitkreutz BJ, Tyers M.** 2002. Systematic identification of pathways that couple cell growth and division in yeast. Science **297:**395–400. http://dx.doi.org/10.1126/science.1070850.
5. **Adams J, Hansche PE.** 1974. Population studies in microorganisms. I. Evolution of diploidy in *Saccharomyces cerevisiae*. Genetics **76:**327–338.
6. **de Godoy LM, Olsen JV, Cox J, Nielsen ML, Hubner NC, Fröhlich F, Walther TC, Mann M.** 2008. Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. Nature **455:**1251–1254. http://dx.doi.org/10.1038/nature07341.
7. **McMurrough I, Rose AH.** 1967. Effect of growth rate and substrate limitation on the composition and structure of the cell wall of *Saccharomyces cerevisiae*. Biochem. J. **105:**189–203.
8. **Johnston GC, Ehrhardt CW, Lorincz A, Carter BL.** 1979. Regulation of cell size in the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **137:**1–5.
9. **Tyson CB, Lord PG, Wheals AE.** 1979. Dependency of size of *Saccharomyces cerevisiae* cells on growth rate. J. Bacteriol. **138:**92–98.
10. **Zakrzewska A, van Eikenhorst G, Burggraaff JE, Vis DJ, Hoefsloot H, Delneri D, Oliver SG, Brul S, Smits GJ.** 2011. Genome-wide analysis of yeast stress survival and tolerance acquisition to analyze the central trade-off between growth rate and cellular robustness. Mol. Biol. Cell **22:**4435–4446. http://dx.doi.org/10.1091/mbc.E10-08-0721.
11. **de Nobel JG, Klis FM, Priem J, Munnik T, van den Ende H.** 1990. The glucanase-soluble mannoproteins limit cell wall porosity in *Saccharomyces cerevisiae*. Yeast **6:**491–499. http://dx.doi.org/10.1002/yea.320060606.
12. **Broach JR.** 2012. Nutritional control of growth and development in yeast. Genetics **192:**73–105. http://dx.doi.org/10.1534/genetics.111.135731.
13. **Schaber J, Adrover MA, Eriksson E, Pelet S, Petelenz-Kurdziel E, Klein D, Posas F, Goksör M, Peter M, Hohmann S, Klipp E.** 2010. Biophysical properties of *Saccharomyces cerevisiae* and their relationship with HOG pathway activation. Eur. Biophys. J. **39:**1547–1556. http://dx.doi.org/10.1007/s00249-010-0612-0.
14. **Morris GJ, Winters L, Coulson GE, Clarke KJ.** 1986. Effect of osmotic stress on the ultrastructure and viability of the yeast *Saccharomyces cerevisiae*. J. Gen. Microbiol. **132:**2023–2034.
15. **Martinez de Marañon I, Marechal P-A, Gervais P.** 1996. Passive response of *Saccharomyces cerevisiae* to osmotic shifts: cell volume variations depending on the physiological state. Biochem. Biophys. Res. Commun. **227:**519–523. http://dx.doi.org/10.1006/bbrc.1996.1539.
16. **De Nobel JG, Klis FM, Munnik T, Priem J, Van Den Ende H.** 1990. An assay of relative cell wall porosity in *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Schizosaccharomyces pombe*. Yeast **6:**483–490. http://dx.doi.org/10.1002/yea.320060605.
17. **Ene IV, Adya AK, Wehmeier S, Brand AC, MacCallum DM, Gow NA, Brown AJ.** 2012. Host carbon sources modulate cell wall architecture, drug resistance and virulence in a fungal pathogen. Cell. Microbiol. **14:**1319–1335. http://dx.doi.org/10.1111/j.1462-5822.2012.01813.x.
18. **Arnold WN, Lacy JS.** 1977. Permeability of the cell envelope and osmotic behavior in *Saccharomyces cerevisiae*. J. Bacteriol. **131:**564–571.
19. **Sevilla MJ, Odds FC.** 1986. Development of *Candida albicans* hyphae in different growth media—variations in growth rates, cell dimensions and timing of morphogenetic events. J. Gen. Microbiol. **132:**3083–3088.
20. **Chaffin WL.** 1984. The relationship between yeast cell size and cell division in *Candida albicans*. Can. J. Microbiol. **30:**192–203. http://dx.doi.org/10.1139/m84-030.
21. **Hartwell LH, Unger MW.** 1977. Unequal division in *Saccharomyces cerevisiae* and its implications for the control of cell division. J. Cell Biol. **75:**422–435. http://dx.doi.org/10.1083/jcb.75.2.422.
22. **Kocková-Kratochvílová A.** 1990. Yeasts and yeast-like organisms, p 29. VCH Verlagsgesellschaft mbH Weinheim, Basel, Switzerand.
23. **Powell CD, Quain DE, Smart KA.** 2003. Chitin scar breaks in aged *Saccharomyces cerevisiae*. Microbiology **149:**3129–3137. http://dx.doi.org/10.1099/mic.0.25940-0.
24. **Sudbery P, Gow N, Berman J.** 2004. The distinct morphogenic states of *Candida albicans*. Trends Microbiol. **12:**317–324. http://dx.doi.org/10.1016/j.tim.2004.05.008.
25. **Gooday GW, Gow NAR.** 1983. A model of the hyphal septum of *Candida albicans*. Exp. Mycol. **7:**370–373. http://dx.doi.org/10.1016/0147-5975(83)90021-X.
26. **Hickman MA, Zeng G, Forche A, Hirakawa MP, Abbey D, Harrison BD, Wang YM, Su CH, Bennett RJ, Wang Y, Berman J.** 2013. The 'obligate diploid' *Candida albicans* forms mating-competent haploids. Nature **494:**55–59. http://dx.doi.org/10.1038/nature11865.
27. **Soll DR, Herman MA.** 1983. Growth and the inducibility of mycelium formation in *Candida albicans*: a single-cell analysis using a perfusion chamber. J. Gen. Microbiol. **129:**2809–2824.
28. **Gow NA, Gooday GW.** 1982. Vacuolation, branch production and linear growth of germ tubes in Candida albicans. J. Gen. Microbiol. **128:**2195–2198.
29. **Gow NAR, Gooday GW.** 1982. Growth kinetics and morphology of

colonies of the filamentous form of *Candida albicans*. J. Gen. Microbiol. **128:**2187–2194.
30. **Fradin C, Slomianny MC, Mille C, Masset A, Robert R, Sendid B, Ernst JF, Michalski JC, Poulain D.** 2008. Beta-1,2 oligomannose adhesin epitopes are widely distributed over the different families of *Candida albicans* cell wall mannoproteins and are associated through both N- and O-glycosylation processes. Infect. Immun. **76:**4509–4517. http://dx.doi.org/10.1128/IAI.00368-08.
31. **Klis FM, Sosinska GJ, de Groot PW, Brul S.** 2009. Covalently linked cell wall proteins of *Candida albicans* and their role in fitness and virulence. FEMS Yeast Res. **9:**1013–1028. http://dx.doi.org/10.1111/j.1567-1364.2009.00541.x.
32. **Orlean P.** 2012. Architecture and biosynthesis of the *Saccharomyces cerevisiae* cell wall. Genetics **192:**775–818.
33. **Klis FM, Brul S, De Groot PW.** 2010. Covalently linked wall proteins in ascomycetous fungi. Yeast **27:**489–493. http://dx.doi.org/10.1002/yea.1747.
34. **Chaffin WL.** 2008. *Candida albicans* cell wall proteins. Microbiol. Mol. Biol. Rev. **72:**495–544. http://dx.doi.org/10.1128/MMBR.00032-07.
35. **Moreno-Ruiz E, Ortu G, de Groot PW, Cottier F, Loussert C, Prévost MC, de Koster C, Klis FM, Goyard S, d'Enfert C.** 2009. The GPI-modified proteins Pga59 and Pga62 of Candida albicans are required for cell wall integrity. Microbiology **155:**2004–2020. http://dx.doi.org/10.1099/mic.0.028902-0.
36. **Biswas SK, Yamaguchi M, Naoe N, Takashima T, Takeo K.** 2003. Quantitative three-dimensional structural analysis of *Exophiala dermatitidis* yeast cells by freeze-substitution and serial ultrathin sectioning. J. Electron Microsc. (Tokyo) **52:**133–143. http://dx.doi.org/10.1093/jmicro/52.2.133.
37. **Yamaguchi M, Biswas SK, Suzuki Y, Furukawa H, Takeo K.** 2003. Three-dimensional reconstruction of a pathogenic yeast *Exophiala dermatitidis* cell by freeze-substitution and serial sectioning electron microscopy. FEMS Microbiol. Lett. **219:**17–21. http://dx.doi.org/10.1016/S0378-1097(02)01181-3.
38. **Klis FM, Boorsma A, De Groot PW.** 2006. Cell wall construction in *Saccharomyces cerevisiae*. Yeast **23:**185–202. http://dx.doi.org/10.1002/yea.1349.
39. **Heilmann CJ, Sorgo AG, Klis FM.** 2012. News from the fungal front: wall proteome dynamics and host-pathogen interplay. PLoS Pathog. **8:**e1003050. http://dx.doi.org/10.1371/journal.ppat.1003050.
40. **François JM.** 2006. A simple method for quantitative determination of polysaccharides in fungal cell walls. Nat. Protoc. **1:**2995–3000. http://dx.doi.org/10.1038/nprot.2006.457.
41. **Levin DE.** 2011. Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway. Genetics **189:**1145–1175. http://dx.doi.org/10.1534/genetics.111.128264.
42. **Kapteyn JC, Hoyer LL, Hecht JE, Müller WH, Andel A, Verkleij AJ, Makarow M, Van Den Ende H, Klis FM.** 2000. The cell wall architecture of *Candida albicans* wild-type cells and cell wall-defective mutants. Mol. Microbiol. **35:**601–611. http://dx.doi.org/10.1046/j.1365-2958.2000.01729.x.
43. **Munro CA, Richard ML.** 2012. The cell wall: glycoproteins, remodeling, and regulation, p 197–223. *In* Calderone RA, Clancy CJ (ed), Candida and candidiasis, 2nd ed. ASM Press, Washington, DC.
44. **Sosinska GJ, de Koning LJ, de Groot PW, Manders EM, Dekker HL, Hellingwerf KJ, de Koster CG, Klis FM.** 2011. Mass spectrometric quantification of the adaptations in the wall proteome of *Candida albicans* in response to ambient pH. Microbiology **157:**136–146. http://dx.doi.org/10.1099/mic.0.044206-0.
45. **Sosinska GJ, de Groot PW, Teixeira de Mattos MJ, Dekker HL, de Koster CG, Hellingwerf KJ, Klis FM.** 2008. Hypoxic conditions and iron restriction affect the cell-wall proteome of *Candida albicans* grown under vagina-simulative conditions. Microbiology **154:**510–520. http://dx.doi.org/10.1099/mic.0.2007/012617-0.
46. **Sorgo AG, Heilmann CJ, Dekker HL, Bekker M, Brul S, de Koster CG, de Koning LJ, Klis FM.** 2011. Effects of fluconazole on the secretome, the wall proteome, and wall integrity of the clinical fungus *Candida albicans*. Eukaryot. Cell **10:**1071–1081. http://dx.doi.org/10.1128/EC.05011-11.
47. **Sorgo AG, Brul S, de Koster CG, de Koning LJ, Klis FM.** 2013. Iron restriction-induced adaptations in the wall proteome of *Candida albicans*. Microbiology **159:**1673–1682. http://dx.doi.org/10.1099/mic.0.065599-0.
48. **Heilmann CJ, Sorgo AG, Mohammadi S, Sosinska GJ, de Koster CG, Brul S, de Koning LJ, Klis FM.** 2013. Surface stress induces a conserved cell wall stress response in the pathogenic fungus *Candida albicans*. Eukaryot. Cell **12:**254–264. http://dx.doi.org/10.1128/EC.00278-12.
49. **Ene IV, Heilmann CJ, Sorgo AG, Walker LA, de Koster CG, Munro CA, Klis FM, Brown AJ.** 2012. Carbon source-induced reprogramming of the cell wall proteome and secretome modulates the adherence and drug resistance of the fungal pathogen *Candida albicans*. Proteomics **12:**3164–3179. http://dx.doi.org/10.1002/pmic.201200228.
50. **Heilmann CJ, Sorgo AG, Siliakus AR, Dekker HL, Brul S, de Koster CG, de Koning LJ, Klis FM.** 2011. Hyphal induction in the human fungal pathogen *Candida albicans* reveals a characteristic wall protein profile. Microbiology **157:**2297–2307. http://dx.doi.org/10.1099/mic.0.049395-0.
51. **de Groot PW, Bader O, de Boer AD, Weig M, Chauhan N.** 2013. Adhesins in human fungal pathogens: glue with plenty of stick. Eukaryot. Cell **12:**470–481. http://dx.doi.org/10.1128/EC.00364-12.
52. **Cassone A, Simonetti N, Strippoli V.** 1973. Ultrastructural changes in the wall during germ-tube formation from blastospores of *Candida albicans*. J. Gen. Microbiol. **77:**417–426. http://dx.doi.org/10.1099/00221287-77-2-417.
53. **Dupres V, Dufrêne YF, Heinisch JJ.** 2010. Measuring cell wall thickness in living yeast cells using single molecular rulers. ACS Nano **4:**5498–5504. http://dx.doi.org/10.1021/nn101598v.
54. **Kocková-Kratochvílová A.** 1990. Yeasts and yeast-like organisms, p 35. VCH Verlagsgesellschaft mbH Weinheim, Basel, Switzerland.
55. **Dague E, Bitar R, Ranchon H, Durand F, Yken HM, François JM.** 2010. An atomic force microscopy analysis of yeast mutants defective in cell wall architecture. Yeast **27:**673–684. http://dx.doi.org/10.1002/yea.1801.
56. **Mouyna I, Fontaine T, Vai M, Monod M, Fonzi WA, Diaquin M, Popolo L, Hartland RP, Latgé JP.** 2000. Glycosylphosphatidylinositol-anchored glucanosyltransferases play an active role in the biosynthesis of the fungal cell wall. J. Biol. Chem. **275:**14882–14889. http://dx.doi.org/10.1074/jbc.275.20.14882.
57. **Cabib E, Arroyo J.** 2013. How carbohydrates sculpt cells: chemical control of morphogenesis in the yeast cell wall. Nat. Rev. Microbiol. **11:**648–655. http://dx.doi.org/10.1038/nrmicro3090.
58. **Alsteens D, Beaussart A, El-Kirat-Chatel S, Sullan RMA, Dufrêne YF.** 2013. Atomic force microscopy: a new look at pathogens. PLoS Pathog. **9:**e1003516. http://dx.doi.org/10.1371/journal.ppat.1003516.
59. **Scherrer R, Louden L, Gerhardt P.** 1974. Porosity of the yeast cell wall and membrane. J. Bacteriol. **118:**534–540.
60. **Cope JE.** 1980. The porosity of the cell wall of *Candida albicans*. J. Gen. Microbiol. **119:**253–255.
61. **De Nobel JG, Dijkers C, Hooijberg E, Klis FM.** 1989. Increased cell wall porosity in *Saccharomyces cerevisiae* after treatment with dithiothreitol or EDTA. J. Gen. Microbiol. **135:**2017–2084.
62. **de Groot PW, Kraneveld EA, Yin QY, Dekker HL, Gross U, Crielaard W, de Koster CG, Bader O, Klis FM, Weig M.** 2008. The cell wall of the human pathogen *Candida glabrata*: differential incorporation of novel adhesin-like wall proteins. Eukaryot. Cell **7:**1951–1964. http://dx.doi.org/10.1128/EC.00284-08.
63. **Ragni E, Sipiczki M, Strahl S.** 2007. Characterization of Ccw12p, a major key player in cell wall stability of *Saccharomyces cerevisiae*. Yeast **24:**309–319. http://dx.doi.org/10.1002/yea.1465.
64. **Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS.** 2003. Global analysis of protein expression in yeast. Nature **425:**737–741. http://dx.doi.org/10.1038/nature02046.
65. **Erickson HP.** 2009. Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. Biol. Proced. Online **11:**32–51. http://dx.doi.org/10.1007/s12575-009-9008-x.
66. **Smits GJ, Schenkman LR, Brul S, Pringle JR, Klis FM.** 2006. Role of cell cycle-regulated expression in the localized incorporation of cell wall proteins in yeast. Mol. Biol. Cell **17:**3267–3280.
67. **Yin QY, de Groot PW, de Jong L, Klis FM, De Koster CG.** 2007. Mass spectrometric quantitation of covalently bound cell wall proteins in *Saccharomyces cerevisiae*. FEMS Yeast Res. **7:**887–896. http://dx.doi.org/10.1111/j.1567-1364.2007.00272.x.
68. **Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N, Pillai S, Dey S, Daniels S, Purkayastha S, Juhasz P, Martin S, Bartlet-Jones M, He F, Jacobson A, Pappin DJ.** 2004. Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. Mol. Cell. Proteomics **3:**1154–1169. http://dx.doi.org/10.1074/mcp.M400129-MCP200.
69. **Krátký Z, Biely P, Bauer S.** 1975. Wall mannan of *Saccharomyces cerevi-*

siae. Metabolic stability and release into growth medium. Biochim. Biophys. Acta **404:**1–6.
70. **Pastor FI, Herrero E, Sentandreu R.** 1982. Metabolism of *Saccharomyces cerevisiae* envelope mannoproteins. Arch. Microbiol. **132:**144–148. http://dx.doi.org/10.1007/BF00508720.
71. **Tkacz JS, Lampen JO.** 1972. Wall replication in *Saccharomyces* species: use of fluorescein-conjugated concanavalin A to reveal the site of mannan insertion. J. Gen. Microbiol. **72:**243–247. http://dx.doi.org/10.1099/00221287-72-2-243.
72. **Sorgo AG, Heilmann CJ, Dekker HL, Brul S, de Koster CG, Klis FM.** 2010. Mass spectrometric analysis of the secretome of Candida albicans. Yeast **27:**661–672. http://dx.doi.org/10.1002/yea.1775.
73. **Sorgo AG, Heilmann CJ, Brul S, de Koster CG, Klis FM.** 2013. Beyond the wall: *Candida albicans* secret(e)s to survive. FEMS Microbiol. Lett. **338:**10–17. http://dx.doi.org/10.1111/1574-6968.12049.
74. **Ertugay N, Hamamci H.** 1997. Continuous cultivation of bakers' yeast: change in cell composition at different dilution rates and effect of heat stress on trehalose level. Folia Microbiol. (Praha) **42:**463–467. http://dx.doi.org/10.1007/BF02826554.
75. **Coleman DA, Oh SH, Zhao X, Hoyer LL.** 2010. Heterogeneous distribution of *Candida albicans* cell-surface antigens demonstrated with an Als1-specific monoclonal antibody. Microbiology **156:**3645–3659. http://dx.doi.org/10.1099/mic.0.043851-0.
76. **Albuquerque PC, Nakayasu ES, Rodrigues ML, Frases S, Casadevall A, Zancope-Oliveira RM, Almeida IC, Nosanchuk JD.** 2008. Vesicular transport in *Histoplasma capsulatum*: an effective mechanism for transcell wall transfer of proteins and lipids in ascomycetes. Cell. Microbiol. **10:**1695–1710. http://dx.doi.org/10.1111/j.1462-5822.2008.01160.x.
77. **Mitchison JM.** 1958. The growth of single cells. II. *Saccharomyces cerevisiae*. Exp. Cell Res. **15:**214–221.
78. **Herman MA, Soll DR.** 1984. A comparison of volume growth during bud and mycelium formation in *Candida albicans*: a single cell analysis. J. Gen. Microbiol. **130:**2219–2228.
79. **Karpova TS, Reck-Peterson SL, Elkind NB, Mooseker MS, Novick PJ, Cooper JA.** 2000. Role of actin and Myo2p in polarized secretion and growth of *Saccharomyces cerevisiae*. Mol. Biol. Cell **11:**1727–1737. http://dx.doi.org/10.1091/mbc.11.5.1727.
80. **López-Franco R, Bartnicki-Garcia S, Bracker CE.** 1994. Pulsed growth of fungal hyphal tips. Proc. Natl. Acad. Sci. U. S. A. **91:**12228–12232. http://dx.doi.org/10.1073/pnas.91.25.12228.
81. **Gooday GW.** 1995. The dynamics of hyphal growth. Mycol. Res. **99:**385–394.
82. **Lorenz MC, Bender JA, Fink GR.** 2004. Transcriptional response of *Candida albicans* upon internalization by macrophages. Eukaryot. Cell **3:**1076–1087. http://dx.doi.org/10.1128/EC.3.5.1076-1087.2004.
83. **Munro CA, Selvaggini S, de Bruijn I, Walker L, Lenardon MD, Gerssen B, Milne S, Brown AJ, Gow NA.** 2007. The PKC, HOG and Ca2+ signalling pathways co-ordinately regulate chitin synthesis in *Candida albicans*. Mol. Microbiol. **63:**1399–1413. http://dx.doi.org/10.1111/j.1365-2958.2007.05588.x.
84. **Jacobson K, Mouritsen OG, Anderson RG.** 2007. Lipid rafts: at a crossroad between cell biology and physics. Nat. Cell Biol. **9:**7–14. http://dx.doi.org/10.1038/ncb0107-7.
85. **Backhaus K, Heilmann CJ, Sorgo AG, Purschke G, de Koster CG, Klis FM, Heinisch JJ.** 2010. A systematic study of the cell wall composition of *Kluyveromyces lactis*. Yeast **27:**647–660. http://dx.doi.org/10.1002/yea.1781.
86. **Magnelli PE, Cipollo JF, Robbins PW.** 2005. A glucanase-driven fractionation allows redefinition of *Schizosaccharomyces pombe* cell wall composition and structure: assignment of diglucan. Anal. Biochem. **336:**202–212. http://dx.doi.org/10.1016/j.ab.2004.09.022.
87. **de Groot PW, Yin QY, Weig M, Sosinska GJ, Klis FM, de Koster CG.** 2007. Mass spectrometric identification of covalently bound cell wall proteins from the fission yeast *Schizosaccharomyces pombe*. Yeast **24:**267–278. http://dx.doi.org/10.1002/yea.1443.
88. **Baldwin WW, Kubitschek HE.** 1984. Buoyant density variation during the cell cycle of *Saccharomyces cerevisiae*. J. Bacteriol. **158:**701–704.
89. **Kocková-Kratochvílová A.** 1990. Yeasts and yeast-like organisms, p 134. VCH Verlagsgesellschaft mbH Weinheim, Basel, Switzerland.
90. **Fleet GH, Manners DJ.** 1976. Isolation and composition of an alkali-soluble glucan from the cell walls of *Saccharomyces cerevisiae*. J. Gen. Microbiol. **94:**180–192. http://dx.doi.org/10.1099/00221287-94-1-180.
91. **Boone C, Sommer SS, Hensel A, Bussey H.** 1990. Yeast KRE genes provide evidence for a pathway of cell wall beta-glucan assembly. J. Cell Biol. **110:**1833–1843. http://dx.doi.org/10.1083/jcb.110.5.1833.